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THE JOURNAL OF HYGIENE

CAMBRIDGE UNIVERSITY PRESS

London: FETTER LANE, E.C.

C. F. CLAY, MANAGER



Edinburgh: 100, PRINCES STREET

London: H. K. LEWIS, 136, GOWER STREET, W.C.

WILLIAM WESLEY AND SON, 28, ESSEX STREET, STRAND

Berlin: A. ASHER AND CO.

Leipzig: F. A. BROCKHAUS

Chicago: THE UNIVERSITY OF CHICAGO PRESS

Bombay and Calcutta: MACMILLAN AND CO., LTD.

Toronto: J. M. DENT AND SONS, LTD.

Tokyo: THE MARUZEN-KABUSHIKI-KAISHA

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C. 1
P. 112 / 4

THE
JOURNAL OF HYGIENE

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Volume XIII. 1913-14

Cambridge
at the University Press
1914

13 2925
2/6/14

RA
421
J88
v.13
cop. 2

Cambridge:

PRINTED BY JOHN CLAY, M.A.

AT THE UNIVERSITY PRESS

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AN ACCOUNT OF A GLANDERS-LIKE DISEASE
OCCURRING IN RANGOON.

By A. WHITMORE, M.D. (Cantab.).

(With Plate I and 2 Charts.)

THE opportunities of a pathologist at a large Eastern Hospital are many; but his time for research work is short, and his conveniences are few. I, therefore, feel that we, at the Laboratory of the Rangoon General Hospital, are more than usually fortunate to be able to give, within the first two years of the official existence of the Laboratory, an account of a strange disease. Doubtless it is to chance that we owe the first discrimination of the disease; but I hope that it has been by accurate observation and precise experiment that we have sought to fulfil our knowledge. The guidance and application of our observations have been my care, and upon me rests the responsibility for the accuracy of our work; but the work itself has been carried out entirely by my assistants, and to them belongs the credit for the zeal and patient perseverance with which the task has been performed.

About one and a half years ago we had occasion to report to the Health Authorities of Rangoon the discovery of a few cases of human glanders infection; the opportunity was then seized for a much-needed effort to lessen the prevalence of this infection among the "gharry" ponies of the town. The difficulties and opposition met with by the authorities in carrying out their sanitary measures so excited our sympathy, and stimulated our interest, that our zeal in the detection of the infection was not allowed to wane. In the course of these bacteriological investigations our eyes were opened to the presence among the ill-nourished and neglected inhabitants of the town of a strange infective disease somewhat resembling glanders.

In April, 1911, we performed a post-mortem examination upon the body of a Burman aged 40 years (Case 1). He had been admitted to the hospital for fever of seven days' duration and died three days after

admission; during these three days' stay in hospital his temperature had been high, ranging from 103° to 104° F.: he was a morphia injector: for his thighs were covered with the marks of injections, and in connection with these there were several superficial abscesses.

At the post-mortem examination the principal lesion discovered was a peculiar cheesy consolidation of the lungs. The distribution and appearance of this consolidation were those of neither lobar pneumonia nor tubercular infection, and upon examining smears from the diseased patches a large number of non-Gram staining bacilli, of the size and shape of *Bacillus mallei*, were seen to be present. As our minds were then intent upon the detection of glanders, it was not strange that we made a preliminary diagnosis of this infection. We notified the Medical Officer of Health of our suspicion, and he replied that a glanders infection appeared improbable: as, so far as he could discover, the man had been released from the jail only a short time previously and had had no close contact with horses. In the meantime we had made cultures from the diseased lung: these cultures upon ordinary peptone agar gave luxuriant growths, and, upon examining the growths after three days' incubation, we found that they consisted of pure cultures of what appeared to be non-motile bacilli of the size, and shape, of those which we had found in the lung smears. We were rather puzzled by the rapidity, and luxuriance, of the growth, otherwise we were quite satisfied that the bacillus would turn out to be *B. mallei*; and it was without any misgivings as to the results that we passed on to carry out the cultural and inoculation tests characteristic of this bacillus.

After 24 hours an inoculation of a potato slope gave a lightish yellow growth which, although rather rapid and luxuriant, was otherwise not unlike the growth to be expected from an inoculation with *B. mallei*. 20 minims of a 24 hours' broth culture were injected intraperitoneally into a male guinea-pig. To our disappointment, the guinea-pig died within 36 hours without any obvious inflammation of the testicles. The post-mortem examination disclosed no signs of very acute peritonitis, although a small amount of free fluid was present within the peritoneal cavity: the omentum was rolled up, and along the rolled up, matted omentum were deposits of acute inflammatory lymph; there was also acute peri-hepatitis, but the spleen appeared to be normal. Smears from the general peritoneal cavity showed a few bacilli, in the matted omentum these were very numerous, in the liver smears they were present in moderate numbers; while in the spleen smears one or two only could be seen. From the omentum, liver, and spleen, pure

growths of a very actively motile bacillus were obtained. As we were under the impression that the bacilli obtained from the diseased lung were non-motile, we were at first inclined to the opinion that the bacilli isolated from this guinea-pig were not of the species of those inoculated, but belonged to that of *B. coli*; an injury of the intestine at the time of operation having provided a possibility for this infection. We would, I think, have abandoned this experiment as a failure, had it not been that during the past few years we have seen much of the effects of an acutely fatal peritonitis, the result of bowel injury. For in considering the signs of peritonitis presented by the peritoneal cavity of this guinea-pig, we felt dissatisfied with the simple view that they were due to a "coli" infection, and adopted an alternative view, viz. that the bacillus isolated from the human lung, though at first motile, might lose its motility after a few days' cultivation upon artificial media. This view proved to be correct; for it was found, not only that bacilli in the cultures from the guinea-pig had almost completely lost their very active motility after a few days' cultivation, but also that the bacilli in young subcultures from our original lung cultures had become actively motile. It now seemed possible that the bacilli which had caused the death of the guinea-pig, and those which had been isolated from the diseased lung, were of one and the same species; but if so it was plain that we were dealing, not with *B. mallei*, but with some other organism with which we were up to that time unacquainted; and that therefore, if, as seemed probable, the disease in the lung was the result of an infection by this bacillus, it was a disease so far unfamiliar to us. Further experience alone could decide this question; nor had we long to wait for such experience. A Burman, aged 30 years, with a history of having suffered from fever for about a month, and from dysentery during the last week, was admitted in a moribund condition to hospital: he died after less than 24 hours' stay (Case 2).

Post-mortem notes:

"An emaciated body with numerous marks of morphia injections.

"Lungs: scattered throughout both lobes of the left lung were numerous patches of the peculiar and characteristic consolidation; while in the right lung there were a fair number of such patches in the upper lobe, and a few in the lower lobe.

"The spleen was soft, and twice the normal size.

"The large bowel was extensively ulcerated: the ulcers having the appearances usual in those due to amoebic dysentery.

"The other organs of the body were normal."

Cultures from both lungs and spleen of this case gave luxuriant growths, in pure culture, of the bacillus under investigation.

Animal inoculations with the bacilli isolated from these two cases were again undertaken. Male guinea-pigs were inoculated both intraperitoneally and subcutaneously with young broth cultures, and similar results were obtained with both strains of the bacillus. The guinea-pigs inoculated intraperitoneally died, or rather were moribund, within 48 hours; and in contrast with the effects of our first animal inoculation there was, in both these animals, an obvious inflammatory enlargement of the testicles; which at the post-mortem examination was seen to be due to an acute infection of the tunica vaginalis. The abdominal cavity and its contents presented similar changes to those noted in our first inoculation; and in addition, in these two cases, the spleen was enlarged and studded with numerous small white points of inflammatory deposits. Along the needle track of inoculation a sort of caseous infiltration with a haemorrhagic surrounding gave evidence of acute inflammation of the abdominal walls. The lungs were normal.

The animals inoculated subcutaneously died more slowly; in from three to four days. Within ten hours the tissues around the sites of inoculation became widely infiltrated, and inflamed; and after death the most important change noted was the extensive matting of the tissues around the inoculation sites. This matting was due to caseous, inflammatory material, and very little fluid pus was present. In both cases there was an obvious enlargement of the spleen; which organ contained numerous tiny, white, inflammatory deposits.

From the diseased organs and tissues of all these four animals pure cultures of the bacilli were obtained.

The similarity of these two cases in their pathological lesions and bacteriological findings compelled the supposition that we had to deal with a definite and unfamiliar infective disease; if this supposition were correct, it seemed reasonable to expect that cases of the infection must have been previously met with among the numerous post-mortem examinations, which we carry out here every year; and that the records of these examinations, if kept as carefully as we hoped, would afford us satisfactory evidence upon this point. We were not disappointed; for upon referring to the records of the previous six months we were able to find some four or five cases, in which lesions of the lungs similar to those present in the above two cases had been noted. In one of these cases we had at first actually returned the case as almost certainly one of glanders infection; but later upon bacteriological investigation we

had failed to confirm this provisional diagnosis: the body had been moderately decomposed at the time of examination, and consequently the motile bacillus isolated had been classed as a product of decomposition; and our failure to isolate the slow growing *B. mallei* explained by the difficulties caused by its association with this very rapidly growing putrefactive bacillus. However, in two other cases we had persevered with our bacteriological researches for some considerable time, and had carefully noted our findings; so that we were able now to resume these researches with a bacteriological knowledge already well founded.

The conviction, that we were justified in our hypothesis that we were dealing with a strange disease, brought us face to face with a problem very different from that in which we had been previously engaged. Until then we had accepted the lung lesions as those not unusual in glanders infection, and had been employed in the simple task of substantiating this diagnosis by bacteriological investigations upon well-known lines. Now, however, our first and most obvious business was to endeavour to ascertain, whether any such disease as this, though unfamiliar to us, had been discovered and described by others. The circumstances of our work are such that it has been impossible for us to refer to literature of wider scope than that of the ordinary current text-books; from these we could obtain no hint that an infective disease of this character had been hitherto described. Therefore, so far as we were concerned, we had now to elucidate an entirely new disease: it was for us, not only to single out the characteristic features of a new bacillus; but also to describe the symptoms set up by its infection of man, to discover their underlying lesions; and to discern the method and incidence of such infections.

During the past year the material, and time, at our disposal have permitted us to satisfactorily fulfil the first portion of the task; but of the second the probable results have been only dimly indicated. From 38 subjects we have isolated bacilli with similar characteristics: in the majority of instances these bacilli alone have been isolated; in the few examples where mixed cultures have been obtained there have been adequate reasons why such mixed infections could have been expected. With difference of origin, the characteristics of the bacilli have differed slightly; but these differences have been so slight, and the resemblances in all points so close, that there has been no doubt possible, but that from all these 38 cases we have isolated bacilli of one and the same species. It has been a laborious, but by no means a difficult task, to

pick out a few characteristics of the bacillus so peculiar, as to distinguish it from all other pathogenic bacteria; and so easily observed as to be ready guides to its speedy identification in the laboratory.

The bacilli as seen in smears from the lesions of an infected organ are rod-shaped bacilli, about the size and shape of the *B. mallei*: they stain readily with all the usual stains, but are not acid-fast; nor do they retain the stain when stained by Gram's method. Stained with Leishman's stain they show a well-marked bipolar staining; the poles being stained a dark purple, while the bodies are blue.

Growth upon all the usual culture media is rapid and luxuriant; and occurs under both aërobic and anaërobic conditions; though more luxuriantly under the former.

For the first 24 hours growth in broth is not very luxuriant; although as early as the end of the tenth hour of incubation it can be seen as a diffuse, faint haze: after 24 hours there is a general turbidity of the broth and a pellicle begins to form at the surface; this pellicle gradually thickens until at the end of the fourth or fifth day it is a tough, resistant, wrinkled skin.

Upon ordinary peptone agar the growth appears in from 8 to 10 hours, as moist, translucent, slightly raised colonies; in 48 hours these colonies have become opaque and thick, and are of a cream colour. In agar cultures of over a month's growth the colonies are dry, with the middle portions wrinkled, and their colour is brown with a tinge of pink.

Upon salted agar, the salt being $1\frac{1}{2}$ to 2% in strength, growth is slow, and appears as a thin layer rather like a thin coating of white paint. A smear preparation from a salted agar culture shows that the bacilli are growing in dense felted masses, composed of very long sinuous filaments. If the strength of salt is over 2%, growth is so slow, that, in order to secure this curious change in the appearance of the bacilli, it is advisable to inoculate a large number of these, sufficient to form immediately after inoculation a thick, visible streak.

Upon glycerine agar, containing 3 to 5% of glycerine, the growth is rapid and luxuriant, but at the end of the second day the lowest third of the culture begins to acquire a wrinkled appearance; and this wrinkling rapidly extends to the whole growth, until after little more than a week's incubation the growth has become heaped up, and rugose, not unlike a thick growth of tubercle bacilli.

Upon gelatine at a temperature of 18° to 22° C. growth is rather slow, but at the end of the third day of incubation there is a white

streak of visible growth, and beneath this liquefaction of the gelatine slowly takes place. In stab cultures this growth produces very characteristic appearances: at the end of the third day there is a faint white streak of growth along the whole needle track, and at the surface the growth is spread out in the shape of a small white disc: by the fourth or fifth day the gelatine just below this surface disc is obviously liquefying: and by the end of the week, or a little later, the liquefaction has progressed to form a small cup of liquefied gelatine covered by a thick wrinkled pellicle; while along the rest of the inoculation stab is a white line of growth with extremely fine dots distributed in a radiating manner out into the surrounding clear gelatine.

Liquefaction of the gelatine has occurred in every case, but the actual rate of the liquefaction has varied considerably with the different strains of bacilli inoculated.

Upon potato a vigorous growth appears in 24 hours. As a rule at its first appearance the growth has been of a cream colour; but with bacilli from one or two cases the initial colour has been light yellow, not unlike that of young cultures of *B. mallei*, but this yellow colour is very quickly lost. The potato round the growth is not discoloured, nor is there much spread of the growth away from the line of inoculation.

In litmus milk growth readily occurs. For the first three days there is no marked change in reaction, but later the casein is precipitated, a thick white fluffy sediment collects at the bottom of the tube, the whey becomes pink, and a violet coloured seum dotted with white spots is formed on the surface.

The various sugar media have been inoculated with the bacilli, but, though growth is vigorous, there has never been the slightest gas formation in any of these media.

In young cultures upon all media the bacilli are actively motile, but this motility is almost entirely lost as the cultures age. In agar cultures the motility is often so diminished by the beginning of the third day that cultures examined then would be accepted as those of non-motile organisms. In broth cultures the bacilli retain their motility for a longer time, but after the tenth day of incubation they have become practically non-motile.

When present the motility is of a curious serpentine character.

We have no evidence of spore formation under any conditions, but our observations upon this point are meagre.

The cultural and other characteristics upon which we rely for distinguishing this bacillus from other pathogenic bacteria are, I think,

fairly obvious; those which we find of particular utility in the rapid identification of the bacillus are:

1. The rapid and luxuriant growth upon ordinary peptone agar.
2. The wrinkling which occurs so early in the growth upon glycerine agar.
3. The pellicle formation at the surface of broth cultures.
4. The appearance of gelatine stab cultures at the end of the third day, and after one week's growth.
5. The curious, tangled masses of long filamentous bacilli found in cultures upon salted agar.
6. The active serpentine motility of the bacilli in young cultures, and its early disappearance as the cultures age.

When a rough preliminary examination of smears from diseased organs is being made the bipolar nature of the staining with Leishman's stain is exceedingly useful in arousing an early suspicion of the nature of the infection.

For testing the pathogenic effects of the bacilli upon animals we have made use of guinea-pigs only.

Both subcutaneous and intraperitoneal inoculations into these animals have been made, and all strains of bacilli have been found markedly pathogenic. In all our experiments the animal has shown signs of very serious illness within a few days. At first the dosage of bacilli used was very large, 20 minims of a 24 hours' broth culture being injected as a dose, and in these cases the animals were either dead or moribund within 48 hours; but in the later experiments, made for diagnostic purposes, very much smaller doses have been given.

The results of the large inoculations have already been mentioned while describing the investigations into our first two recorded cases of the disease. The guinea-pigs die of a septicaemic disease with well-marked local lesions depending upon the site of infection. With the large intraperitoneal inoculations the animals die so rapidly that the inflammation of the testicles, which occurs in the male pig, may not have time to develop in any very obvious manner; the swelling and redness may be so slight as to be easily overlooked; this no doubt happened in our first experiment. Therefore, if the occurrence of these characteristic local inflammations is to be utilised for diagnostic purposes, a very small dosage is requisite: we have found that half to one minim of an 18 hours' broth culture given, either intraperitoneally, or subcutaneously, is an ample and useful dose. This dose given intraperitoneally to a

male pig causes a very well-marked, and characteristic, Strauss's reaction which is obvious within 36 hours of the injection.

Within the fourth or fifth day from the time of inoculation the guinea-pig is obviously seriously ill, and may die even within five days of injection; however, the exact dates of obvious serious illness and death have varied very considerably with the strains of bacilli used.

A small dose given subcutaneously gives rise to a rapid induration of the tissues around the site of injection, and this infiltration, or induration, is due to a matting of the tissues by a thick caseous exudate; in the caseous matter, which can be easily expressed, the bacilli are extremely numerous, and can be readily demonstrated. This rapid local increase of bacilli after inoculation, and the obvious, accompanying inflammatory exudate into the tissues, are extremely useful in demonstrating the presence of bacilli, when they exist in very small numbers in any infective material.

The onset of serious illness is of course later with a subcutaneous than with an intraperitoneal infection, but in all cases serious and fatal illness has occurred, and, as a rule, has developed within seven days of infection.

A few feeding experiments with contaminated food and drink have been carried out, and, so far as infection of the animals is concerned, have always given positive results. For purposes of clinical diagnosis such experiments are too protracted to be serviceable, yet they have proved of great value as a convincing proof that the bacillus isolated from the lung and other organs of man is the cause of the illness; for by food infection we have succeeded in setting up in guinea-pigs a fatal septicaemic disease characterised by lesions of the lungs, exactly similar to the human lung lesions. We have carried out four such experiments, and in all four cases have caused fatal illnesses; and have been able to demonstrate after death extensive lung consolidations due to this bacillary infection. These experiments will be referred to again when considering the method of infection in man.

For the bacillus whose cultural and pathogenic characters have just been described I propose the name *B. pseudomallei*.

In the first cases of the disease observed by us the lesions of the lungs were so obvious, and striking, that we were not unaturally impelled to the view that we had to deal with a "lung disease," and that it was in the lungs alone that gross evidence of infection was to be expected. It is true that in Case 2, the recovery of bacilli from the spleen proved that, at any rate bacteriologically, the disease was more

widespread; but at that time we failed to appreciate the importance of this observation. However, the isolation of bacilli from the heart's blood in Case 3 afforded further proof of the general nature of the infection, and in subsequent cases we found that the presence of bacilli in the spleen could be generally demonstrated as easily as in the macroscopic lesions of the lungs. We therefore adopted the view that though the disease was essentially a "lung disease," yet, that just as in typhoid fever though the most obvious lesions are confined to the intestine, the infection is general: so also in this disease, with its gross and obvious lesions restricted to the lungs, the local nature of the lesions gave no clue to the possible extent of the infection throughout the body.

This recognition that infection had a distribution in the body beyond that of the usual local lesions led to our examining very carefully other organs of the body for macroscopic evidence of infection, and we were not greatly surprised when gross evidence of infection was discovered in the kidneys of Case 4. Subsequently such lesions were found in both liver and kidney of the subjects of infection; but it was not until the occurrence of Case 9 that we became alive to the truth, that the existence of lung lesions was not an essential feature of the disease. In this case the lungs were quite healthy, and at the post-mortem examination one or two very minute abscesses in the liver, and an enlarged soft spleen, were the only evidence of disease to be found. Such evidence pointed to some septicaemic disease as the cause of death, and in accordance with our ordinary routine we made cultures from the spleen to determine if possible the exact nature of the infection. We had no suspicion whatsoever that the infection might prove to be by this new bacillus, and it was not until the agar cultures had aged sufficiently to show the peculiar wrinkling, that we realised with what bacillus we were probably dealing; once our suspicions were aroused they were very rapidly confirmed by the results, both of subcultures upon suitable media, and of the animal inoculation tests.

By the detection of this case we were at last fully convinced that the disease we were investigating was a septicaemia, and that, although macroscopical lesions in the lungs were the usual, and most obvious, signs of infection, yet their presence was not invariable; so that to properly determine the existence of the infection in any suspected case we should be guided, not only by the presence of lung lesions, but also by the results of a bacteriological examination of the spleen. Our subsequent investigations have given results in full agreement with this

conviction. Cultures from the spleen have been attempted in 26 of the recorded cases: in 21 of these the cultures gave positive results: in the five cases, in which the results were negative so far as the isolation of this particular bacillus was concerned, it is important to note that in three the cultures gave luxuriant growths of a coliform organism, from which through want of experience and skill we may have been unable to separate this particular bacillus, even if present. In Case 12 no note has been made beyond the fact that we failed to isolate the bacillus under investigation; and, as in this case there was severe, and acute, dysenteric ulceration of the large bowel, it is quite possible that here also a growth of coliform organisms confused the issue. In Case 13 streptococci in pure culture were grown from the spleen, and as there would be no difficulty in separating the new, rapidly growing bacillus from an associated growth of slowly growing streptococci, it seems that this case must be accepted as proving that in exceptional cases of infection by this new bacillus a splenic culture may fail to reveal the infection; also it is perhaps possible that even with further experience this detection by splenic culture will prove difficult in cases where the cultures are contaminated with such an organism as *B. coli*. It may indeed happen that the presence of some other organism such as *B. coli* or streptococcus prevents the associated growth of these new bacilli: such a point though unlikely has to be yet investigated.

Up to the present time spleen cultures have succeeded in demonstrating in the absence of positive evidence from the lungs an infection by this new bacillus in four cases: viz. Nos. 14, 15, 16 and 29.

The *lung lesions* caused by this infection have been already described as peculiar. The commonest lesion is a patch of consolidation about the size of a hazel nut, the central portion of this consolidation is pale, and generally soft and cheesy, but not so soft as ordinary tubercular caseation; the outer zone is markedly congested, and the seat of minute haemorrhages. Upon the cut surface of an incised lung the patch stands up slightly above the surrounding healthy lung tissue; and is usually more sharply defined, and drier in appearance, than a patch of ordinary broncho-pneumonic consolidation due to pneumococcal infection. In a few cases there have been patches in which very small portions of the consolidated areas have liquefied, and minute abscesses have been formed; in two cases such suppuration has progressed so as to form quite large cavities; but such cavity formation is rare. Patches of this acute consolidation are distributed irregularly throughout the lungs, and the infection appears to have no predilection for any

particular lobe, or part of a lobe. The relatively small patches of consolidation may coalesce so as to form very large areas of consolidation extending over many square inches, in such cases the individual, small, coalescing areas can frequently be distinguished by their haemorrhagic borders; but in other cases the whole of the extensive consolidation presents a uniform, pale, cheesy appearance. It has seemed to us possible that these extensive areas of cheesy consolidation indicate that the disease has run a somewhat chronic course, and we have provisionally classed them in our records as "chronic" cases, and the other cases as "acute." In favour of such a view is the observation that frequently the bacilli are quite scarce in these large areas, while in the small areas with acute haemorrhagic borders the bacilli are always exceedingly numerous.

Where an area of consolidation lies close to the lung surface the pleura over it is acutely inflamed, and irregularly raised, so as to form an injected, corrugated surface, corresponding in extent with the size of the inflamed patch beneath. There is not as a rule much pleural exudate, and adhesions if present are ill-organised, being simply those due to an acute pleural inflammation. In no cases have we found adhesions so organised as to suggest a chronic inflammation comparable with that so usually due to tubercular infection; nor have we so far met with any pleural exudate sufficient in amount, or of such a character, as to warrant a suggestion of empyema: in Case 4 the exudate was considerable, but it consisted of thick, cheesy material without signs of liquefaction.

The *bronchial lymphatic glands* do not appear to be infected; at any rate, we have never found them obviously enlarged or necrotic; and in Case 26 in which we inoculated media with material from the bronchial glands the tubes remained sterile.

The macroscopic lesions sometimes present in the *liver*, and *kidney*, are very similar in their broad features to those found in the lungs; if due allowance is made for the different consistency of the organs. They consist of small areas of necrotic, caseous material, surrounded by injected haemorrhagic zones; these areas, though as a rule small and widely scattered, may become confluent, and lead to the complete disorganisation of the infected organ; the kidneys of Case 4 are examples of this. Liquefaction of the necrotic areas so as to form abscesses occurs more often in the infection of the liver, or kidney, than in that of the lung.

In the *spleen* minute miliary abscesses may occur; but more frequently enlargement and softening only are the signs of infection of this organ.

Such are the pathological lesions usually set up by this infection, but, as our present experience of the disease has been almost entirely acquired in the mortuary, we are as yet very ignorant of the clinical course of the illness during which such lesions appear. In one case only of the infection has the whole course of the illness been observed by a competent medical observer. The case is Case 27, reported to us by Captain Knapp of the Indian Medical Service, who, puzzled during the patient's lifetime by the clinical course of the illness, recognised at the post-mortem examination that the peculiar lesions present in the lungs were similar to those which he had heard me describe, as occasionally met with in our mortuary subjects. Captain Knapp brought the diseased lungs to me at once, and from the appearance of the lesions present in them I had no difficulty in diagnosing the disease; and later this diagnosis was confirmed by the isolation of the new bacillus in pure culture.

An examination of the notes of this case shows that clinically the illness bore a very close resemblance to acute glanders; in fact upon clinical signs alone a diagnosis of glanders would probably have been made; for this diagnosis was seriously considered by Captain Knapp during the patient's lifetime, and rejected only because of the apparent impossibility of a patient living in the jail having had contact with glanders infection. Pyaemia of unknown origin was the diagnosis made. When it is remembered, that a small dose of this new bacillus, injected intraperitoneally into a male guinea-pig, gives rise to an inflammation of the testicles, as marked as that described by Strauss as so typically a result of an injection of *B. mallei*, that its occurrence could be accepted as a test of the nature of the bacillary infection; then the interest in the clinical resemblance between the two diseases is much increased. Of course it may be objected that we have no proof that the subcutaneous, superficial abscesses so prominent a feature in this case of Captain Knapp's, were caused by this new bacillus; such an objection has weight, particularly in view of the occurrence in Cases Nos. 13, 28, 31 and 33 of superficial abscesses which were due to infections, not by the new bacillus, but by some of the ordinary pus-forming organisms. However, the importance of the presence of abscesses in these four cases is much diminished by the observation that such abscesses were very obviously due to infections through the use of dirty needles for the morphia injections, with the marks of which the patients' thighs were covered; that is to say, the abscesses were symptoms, not of the fatal illnesses, but of comparatively trivial, and obviously added, complaints.

Moreover in Case 28, the two abscesses in the neighbourhood of the ankle, far removed from the sites of morphia injection, were proved to be due to this new bacillus; and afford proof that these bacilli do occasionally cause such abscesses as occurred in this case of Captain Knapp's.

The appearance of the lung lesions in this jail case was that of the class we have provisionally called "chronic," and yet the duration of the patient's illness was only forty days. It is worthy of particular remark that at the time of infection the man was in apparent good health, he had never been addicted to morphia injection, or the taking of opium in any form, and was classed in the jail register as a prisoner in every way fit for full hard labour.

The course of the illness in this the only properly observed, and fully recorded, case of infection strikingly fulfilled the expectations based upon a knowledge of the disease acquired in the laboratory and mortuary; and I think that even with such knowledge only it should not be difficult to suspect the infection reasonably early in the course of the illness; once such suspicion is entertained bacteriological proof ought to be easy. At any rate Case 29 shows that such proof is possible. The patient was a morphia injector, and had been admitted for cellulitis of the scrotum. With adequate treatment of the local disease he did not improve; and shortly before the man's death it was reported to us that an infection with this new bacillus appeared to be possible. A bacteriological examination was at once attempted. Although from the clinical signs the existence of a lung infection appeared probable, yet the man, weak and dying, was coughing up very little sputum; as much as could be collected was sent to the laboratory, and examined, but afforded no evidence of the nature of the infection. A few hours before death blood was drawn from a superficial vein of the arm. This small operation was performed amid circumstances of much difficulty; a delirious patient, a midnight hour, absence of intelligent assistance, are not favourable for accurate bacteriological work; and it is not surprising that blood so drawn had become contaminated, and that in the inoculated culture media diverse organisms grew; amid these organisms we failed to detect this particular new bacillus. However, a small amount of the blood had been drawn into a tube of sodium citrate solution and kept as "citrated" blood. In the morning a few minims of this blood were injected subcutaneously into a guinea-pig. Upon the morning of the third day after inoculation the pig was ill, and at the site of inoculation was a small indurated swelling containing

caseous material; in this caseous matter we were able to demonstrate bacilli in large numbers, and by the inoculation of suitable media to obtain pure luxuriant growths of the new bacillus.

The patient died a few hours after examination, and though the fact that he was a Mahomedan prevented permission for a full post-mortem examination, yet we were able to open the body sufficiently to prove that both lungs were free from infection, but that in both liver and spleen macroscopic evidence of infection existed: from both these organs we obtained pure growths of the expected bacillus.

It is somewhat unfortunate that this, the only case up to the present bacteriologically examined during life, should have been so exceptional as to have no gross lesions in the lungs; for it is reasonable to suppose that, when there are lesions in the lungs, the sputum would be necessarily infected, and would be the material most convenient and profitable for furnishing bacteriological proof of the nature of the infection.

Although this case is satisfactory, in so far as it furnished an excellent example of the possibility of establishing the diagnosis of infection in a living patient, yet it helps but little towards the clear delineation of the clinical picture of the disease. The patient was a hospital inmate for the three concluding days only of his illness, he was delirious and desperately ill, quite unable to give any account of the nature or length of his illness; and his friends, negligent of his welfare, did not return to visit him in hospital. Clinically all that could be said of his illness was, that he, a morphia injector, was dying from the effects of some toxæmic disease; and it was the presence of the marks of morphia injections which alone directed suspicion towards the particular infection. It may be objected that in this case bacteriological examination of the blood furnished a positive result in the very last stage only of the illness, and that had such examination been undertaken earlier it would probably not have been helpful. It is impossible to meet this objection in the present state of our knowledge, but upon the whole it seems more probable that bacilli exist in the blood stream fairly early in the illness, than that such a phenomenon is limited to the terminal stages only: the septicaemic nature of the disease in guinea-pigs lends support to this view. Moreover, it has been already pointed out that in the majority of cases the sputum would be much more likely than a bacteriological examination of the blood to provide a convenient answer to the problem of diagnosis. In the case of Captain Knapp's first prisoner the presence of signs of lung infection upon the day of

admission indicates that such infection is probably a very early event in the course of the disease.

The utility of such a test as Widal's agglutination test for typhoid fever for the early detection of this disease is doubtful. In Case 30 blood serum, collected after death, showed no power whatsoever of agglutinating the bacilli from a young broth culture of the new bacillus; but of course this question cannot be decided by this one experiment.

If simple methods such as a sputum, or blood, examination do not prove fruitful except in the later stages of the illness; then a splenic puncture would, I think, be a useful means of diagnosis.

We have seen that we have had but scanty material from which to acquire a proper clinical understanding of the disease, but when we turn to consider the probable pathways of infection, the evidence at our disposal is still more meagre. At the best an experience drawn from the mortuary only can afford but little more than a suggestion as to the usual portal and method of infection; but more particularly is this true where the mortuary subjects are those of an Eastern hospital, where custom forbids post-mortem examination of any save the wastrels and vagabonds of society, who die in hospital deserted by their relatives and friends, or whose dead bodies are brought in from the streets, without a clue to their abode, or a note as to their illness. It is therefore difficult with our present knowledge to do more than discuss, very briefly, the possible ways in which infection may occur. When we looked upon the disease as primarily a lung infection, we were naturally imbued with the idea that experience would show infection to be by way of the respiratory track, and the disease to be spread by means of infected sputum. Although the later widening of our views by no means forbids the consideration of the possibility of such happenings, it does, I think, make them less exclusively probable. For the recognition of the lung disease as a part only of a widespread malady prevents the prejudice that such lesions probably represent the first footprints of invasion, or that the lungs provide the sole strongholds of attack.

It is a striking and suggestive fact that, of the 38 reported cases of infection, 31 bore marks of morphia injections; and of these 31 persons all save two, viz. Cases 28 and 33, were clearly still addicted to the habit at the time of their last illnesses. The question of a direct connection between these morphia injections and the disease naturally arises. The social status of these friendless wastrels is in itself a guarantee that the injections of morphia are given under the very worst

conditions; but still further proof that all the usual precautions of cleanliness are absent in these administrations of the drug is afforded by the reflection that in very many of the cases the sites of past injections are obviously, and grossly, septic. Yet dirty though the injecting syringes and needles may be it appears improbable that this particular infection is thus conveyed. In several of the infected subjects, whose thighs bore numerous injection marks, we have carefully examined the groin glands, and, though it is not unusual to be able to isolate ordinary pyogenic organisms from these glands, we have never succeeded in isolating this new bacillus; but it is to be borne in mind that this new bacillus does not seem to readily infect the lymphatic system, for in cases of severe lung infection in man, and in the artificial infection of guinea-pigs, the appropriate lymph glands are not obviously infected. Moreover, considering the local reaction which occurs after the subcutaneous injection of the bacilli into guinea-pigs, it is not unreasonable to suppose that in man also a very marked local induration would occur at the site of a cutaneous infection; yet, neither in the majority of morphia injectors, nor in any of the other cases of human infection, has there been any evidence whatsoever of an inflammatory induration, such as could indicate the site of infection through a skin injury. The real explanation of the frequency of this infection among morphia injectors appears to lie in the theory that this morphia habit is so disastrous to the well-being of its victims.

While it is unlikely that infection is by way of cutaneous injuries, we have direct experimental evidence that infection may take place by means of contaminated food and drink. We have already described how pathogenic the bacilli are to guinea-pigs. At first we determined this by inoculation experiments only, but later we fed four guinea-pigs with food or drink contaminated with bacilli; and all four animals quickly succumbed to the infection: in each of the animals after death we found lung lesions exactly similar in character to those so usual in the human infection; but in none were we able to demonstrate any intestinal lesion, nor were we able to recover the bacilli from the intestinal contents; it is therefore possible that infection of these animals occurred through the respiratory, and not through the intestinal tract, though the latter would appear to be the more probable route. However this may be, it will be admitted that, if a disease similar in all respects to that occurring naturally in man can be easily caused in susceptible animals by contamination of their food and drink, then in man also such a method of infection is probable.

One fact not already mentioned has a very important bearing upon the facilities for the occurrence of this contamination: in Cases 4 and 7 a small amount of turbid urine was present in the bladder after death, and when this urine was examined bacteriologically it was found that the turbidity was due to the presence in the urine of vast numbers of these new bacilli. Upon post-mortem evidence alone it is quite impossible to form any opinion as to the prevalence of this bacilluria among cases of the infection; but so far as such evidence goes it appears probable that bacilluria is a marked feature in those cases only in which some gross kidney lesion exists; for in Case 24, in which the post-mortem findings seem to indicate that the infection ran a rapid and acute course, the urine collected after death was free from infection. That only a comparatively slight lesion of the kidney can produce a marked bacilluria is shown by the nature of those lesions present in Case 7. The comparative frequency of kidney lesions in cases of the disease cannot really be estimated by the present series of cases, for in the earlier cases it is quite likely that small lesions were overlooked; however, obvious evidence of kidney infection is noted in seven out of the 38 cases recorded.

It might be reasonably expected that amongst an Eastern community, the habits of whose members are such that the ordinary laws of sanitation are more honoured in the breach than in the observance, an acute infectious disease endowed with those facilities to spread afforded by an infected sputum and urine, would be an exceedingly common and important item in the mortality returns. Yet so far as our experience goes the incidence of this particular disease is very narrow, and its victims comparatively few. Of the 38 cases here recorded 30 were morphia injectors, and four of the remaining eight had other serious debilitating diseases associated with this infection. Only three of the subjects of the disease examined by us were well nourished, and thus suggested that the infection had attacked persons previously in good health and strength. However, in considering the significance of the signs of wasting and debility so usually present, it must not be forgotten that one of the effects of the illness itself may be rapid wasting; such rapid wasting is a striking feature in the illnesses of the artificially infected guinea-pigs. Similarly the significance of the association of the infection with other diseases, such as occurred in 13 of our cases, must not be appreciated without paying due regard to the difficulty of determining in such cases the relative dates of onset of the new bacillary infection and the associated disease.

The probable nature of the effect of morphinism as an adjunct to infection has already been mentioned.

Upon the whole the facts at our disposal support the belief that the bacillus is but slightly virulent towards man, but in estimating the incidence of any disease it would be unwise to attach too great a value to facts collected from a public mortuary; and it may well happen that these first reassuring impressions of the low virulence of the bacillus may be early contradicted as experience ripens. Indeed, in Case 27, reported by Captain Knapp, we have very clear proof that a man enjoying certainly averagely good health, and living under better sanitary conditions than those affecting the majority of his free, but from a sanitary point of view less fortunate, fellows, may succumb to the infection.

It is true that in their practical application the results of these observations are of local interest only, yet the inquiry, from which they have been obtained, has had a scope so wide that our inferences, drawn from an experience so narrow and from facts so few, can do but little more than pave the way for further investigations; it is therefore particularly important that our results should be clearly displayed; for this purpose it is, not only convenient, but necessary that our report should end with a brief review of our present position.

SUMMARY.

Upon 38 separate occasions during the year we have isolated from diseased human organs a bacillus with constant characters, of which some are sufficiently peculiar to distinguish it from all pathogenic bacteria previously known to us.

The pathological lesions, from which such bacilli can be easily isolated in pure culture, are often so peculiar in appearance that this appearance alone would suggest doubts, as to the causation of the lesions by any of the usual pathogenic bacteria; and after a short experience would warrant an anticipatory diagnosis of this particular infection.

If guinea-pigs be fed with food or drink contaminated with pure cultures of this bacillus, the animals speedily die, and after death, not only can the bacilli be easily recovered from various organs, but also lesions are present in every way similar to those found in the human subject.

The human disease, characterised by the constant presence of these bacilli, and by the usual presence of these peculiar lesions, is a pyaemic

or septicaemic disease, attended by symptoms which may bear a very close clinical resemblance to glanders; but the distinction between the two diseases is easy if a proper bacteriological examination be carried out; though confusion would at once arise if Strauss's guinea-pig testicular reaction were allowed to decide the nature of infection.

So far as our present knowledge goes, this new disease is particularly prevalent among morphia injectors; but such prevalence is due rather to the general effects of the morphia habit than to any peculiar exposure of its devotees to infection. We expect that it will be found that, just as in guinea-pigs the infection can be conveyed by contaminated food and drink, so in man such contamination affords the usual method and means of infection.

Moreover the excretion of vast numbers of living bacilli in the urine, and probably in the sputum, of many of the infected persons living amid crowded, insanitary surroundings, makes contamination of food and water inevitable.

Finally, while our clinical knowledge of the disease is so scanty that upon clinical signs only no more than a suspicion of the infection can be entertained; yet our bacteriological knowledge is sufficiently exact, and its application sufficiently easy, to allow such suspicion to be readily confirmed, or denied, by a reference to the pathological laboratory.

Plate I illustrates cultures of the bacillus and the macroscopic appearance of the lung in Case 1. Brief notes of the 38 cases hitherto investigated are added.

The brief records of the thirty-eight cases upon which this paper is based.

Case 1. A Burman, aged 40, admitted to hospital for fever, said to have been of about seven days' duration.

A fairly nourished man, a morphia injector; a few superficial abscesses scattered over the body; temperature fairly high, ranging up to 104° F., moist sounds in both lungs, but no note of any signs of consolidation. He died after three days' stay in hospital. P. M. the lungs contained very numerous patches of the peculiar consolidated areas; smears from these areas showed the bacilli present in large numbers, and a pure culture was obtained with ease: no culture was taken from the spleen or other organ.

Case 2. A Burman, aged 30 years, admitted to hospital in a moribund condition with a history of fever of a month's duration and of lately passing blood in the motions. He died after less than 24 hours' stay in hospital.

P. M. An emaciated body with marks of morphia injection.

Lungs. Left lung, contained numerous patches of a typical appearance throughout both lobes.

Right lung contained numerous similar patches in the upper lobe, with a few patches in the lower lobe.

The spleen was twice the normal size.

The large bowel was covered with gangrenous ulcers of the usual amoebic dysentery type.

Other organs normal.

Culture from both lungs and spleen gave luxuriant growths of the bacillus in pure culture.

Case 3. A Madrassi, male, aged about 45, admitted to hospital for debility and cough; the illness said to have been of one month's duration. He died a few hours after admission.

An emaciated morphia injector.

P.M. performed very shortly after death while the body was still warm.

There were numerous typical consolidated areas in both lungs; also evidence of old tubercular infection, in the shape of a small fibrotic consolidation at the apex of the right lung, and a small thick-walled cavity at the apex of the left lung.

Upon the surface of the spleen were flakes of recent inflammatory lymph.

The groin glands were fairly markedly enlarged.

Cultures were taken from the lungs, spleen, heart's blood and groin glands: pure growths of the bacilli were obtained from the lungs, spleen, and heart's blood, but not from the groin glands.

Case 4. Hindu, male, admitted for pain in the chest and fever; the illness said to have been of one month's duration.

A moderately well-nourished man, very ill, temperature ranging from 99° F. Dulness at the base of the right lung, with increased vocal fremitus; while over both lungs were very numerous bronchitic rales. The patient died three days after admission. Clinical diagnosis 'Lobar Pneumonia' right lung.

P.M. No marks of morphia injection upon the body, which was fairly well nourished.

Lungs. Right, pleura covered with a thick layer of inflammatory lymph, middle lobe of the lung largely consolidated by fluent patches characteristic of this infection: lower lobe a few such patches.

Left lung: a few very small patches of typical consolidation present. Spleen about three times normal in size, and covered with flakes of recent inflammatory lymph. In the interior were one or two minute abscesses.

Kidneys both extensively infected, the kidney substance being riddled with caseous deposits.

In smears from the lungs, kidneys, and urine, were a very large number of bacilli; while a spleen smear showed a few bacilli.

Cultures from the lungs, spleen, and kidneys, gave a luxuriant growth of the bacilli; and from a plate culture of the urine very numerous colonies of the bacilli could be picked out.

Case 5. A Burman, aged about 38 years, found moribund in the street. P.M. An emaciated body with marks of morphia injections upon both thighs.

Lungs. Left lung, a small old cavity at the apex almost certainly tubercular in origin, otherwise lung normal.

In the right lung, there was puckering at the apex, due to old tubercular infection; in lower lobe a very small patch of acute broncho-pneumonia of characteristic appearance was present.

Spleen, old strong adhesions, otherwise normal.

Liver, recent acute adhesions.

Kidneys: in the left were two or three infiltrated patches of a typical character.

The right was normal.

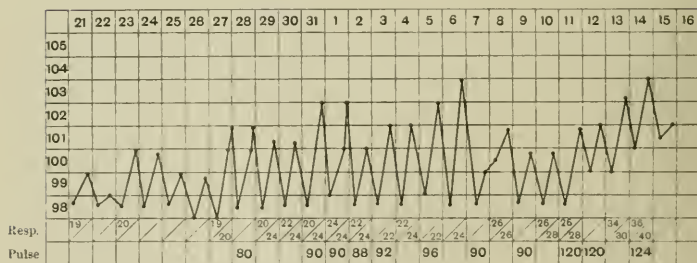
In smears from the lung there were a few acid-fast bacilli.

In smears from the kidney were a fair number of non acid-fast bacilli. Cultures from the kidney patches only were made, and gave pure growths of the bacilli.

Case 6. A Hindu, male, aged 30, admitted to the jail hospital upon arrival, for general debility thought to be the result of chronic "morphinism." The man had irregular fever ranging from normal to 104° F. but had no other very obvious symptoms. Malarial parasites were looked for but not found.

Seven days before death a blood count gave the following differential leucocyte count:

Polynuclears	49 $\frac{0}{10}$
Lymphocytes	8 $\frac{0}{10}$
Mononuclears	37 $\frac{0}{10}$
Eosinophiles	6 $\frac{0}{10}$



Temperature chart of Case 6.

The patient was admitted to the jail hospital upon the 21st of October, 1911, and died November 15th.

The man gradually grew worse, and died twenty-six days after admission to the jail hospital.

P.M. Typical patchy consolidation of the lungs was found, and there were also areas of inflammatory deposits in the liver; the appearance of many of these areas was very similar to that of inflammatory nodules in the lungs, but in one or two places actual suppuration had taken place.

Cultures from both lungs and liver gave pure growths of the bacilli under discussion.

Case 7. Burman, aged about 40 years, picked up in a dying condition, and died very shortly after being brought to hospital.

P.M. A poorly nourished body with fairly numerous marks of morphia injection.

Lungs. Both lungs oedematous, and in the middle lobe of the right lung was one patch of broncho-pneumonia about the size of a pea with the characters of the inflammation caused by this infection.

Spleen, large, and flaccid, covered with old organised adhesions. Kidneys, right healthy, the left contains two small areas of inflammation with characters usual in this disease.

Urine turbid, but no signs of bladder infection or inflammation. Intestine : lower, two-thirds of the large intestine contains fairly numerous old healing ulcers of dysenteric type.

Cultures. From the lung nodule gave a fair number of colonies of the bacillus.

From the kidneys a large number of colonies.

From the spleen about a dozen colonies of the bacillus.

From the urine a fair number of colonies of the bacillus were grown.

Case 8. A Burman, aged about 30 years, picked up dead. A moderately nourished man, with a fair number of morphia injection marks on the right thigh.

P.M. Lungs. Both lungs studded with very numerous nodules ranging in size from a millet seed to a bean, in appearance characteristic of the infection.

Liver surface mottled with small, minute, white dots, and small inflammatory deposits of a typical appearance.

Spleen, slightly enlarged, with fairly numerous, tiny, abscesses. In both kidneys were small deposits characteristic of the infection.

A small amount of turbid urine in the bladder.

In smears from the lung nodules bacilli in fair numbers were seen.

In smears from the spleen a few bacilli were present.

Cultures : liver, a fairly vigorous growth of the bacillus.

Gall-bladder, a coliform bacillus.

Case 9. Hindu, male, aged about 32 years ; dead body picked up in the street.

P.M. A poorly nourished body with slight oedema of the feet, but no marks of morphia injection.

Lungs. No obvious disease in either lung.

Spleen slightly enlarged, about $1\frac{1}{2}$ times normal ; substance soft and friable.

Liver. One or two very minute abscesses, otherwise normal.

A few ankylostoma worms in the intestine.

No other evidence of disease ; death was returned as being probably due to a septicaemic infection of some sort. We did not suspect in any way that it might be this particular infection. However, cultures taken from the spleen gave a luxuriant growth of these bacilli in pure culture. These cultures were tested by animal inoculation.

Case 10. Burman, aged 35 years, admitted to hospital for anorexia and malaria fever of one and a half months' duration. Stay in hospital nine days ; his symptoms being diarrhoea with 6 to 18 stools in the 24 hours, irregular fever, the temperature ranging from 99° F. to 102° F. ; there were signs of acute bronchitis in the lungs, and

the clinical diagnosis was pulmonary tuberculosis, with diarrhoea due to tubercular ulceration of the bowel.

P.M. An emaciated man with the marks of numerous morphia injections.

Lungs. Left lung, one nodule of characteristic appearance in the lower portion of the upper lobe, with a larger nodule of similar appearance in the middle of the lower lobe.

Right lung, one or two characteristic nodules in the lower lobe; the rest of the lung being normal.

The large intestine was full of large acute ulcers of the amoebic dysentery type.

Spleen, normal.

From the lungs were isolated pure growths of the bacilli, but from the spleen these bacilli were not isolated; the spleen cultures growing bacilli of the colon type.

Cultures from the lung patches grew pneumococci and a large coliform bacillus; no new bacilli.

Spleen gave a pure growth of the new bacillus.

Case 11. Hindu, male, aged 25 years, admitted to hospital for looseness of bowels of three months' duration; temperature 101° F.; motions 10 in the twenty-four hours: he died after twenty-four hours in hospital.

P.M. An emaciated young man, not a morphia injector.

Lungs. Right lung normal.

Left lung, a few nodules in the lower lobe characteristic of the infection.

Intestines. The large intestine has numerous ulcers of an amoebic dysentery type chiefly confined to the caecum; the appendix is sloughing in its distal half.

The liver contains one large abscess in the lower part of the right lobe (the usual abscess due to tropical dysentery).

Spleen, normal.

Cultures from the lung gave a vigorous growth of the bacillus.

From the spleen a growth of a coliform bacillus was obtained, but no bacilli of the type under discussion.

Case 12. Dead body of an emaciated Madrassi, aged about 30 years, not a morphia injector, picked up in the street.

P.M. Lungs. Left, upper lobe normal; lower lobe consolidated, with a consolidation like that present in an acute lobar pneumonia, with areas of broncho-pneumonia typical of the new infection scattered through the consolidated portion.

In the right lung, a fair number of dark red consolidated areas probably secondary to a dysenteric infection present.

Spleen twice normal in size; culture negative.

In the kidneys were one or two nodules characteristic of the infection.

Large intestine very extensive, chronic, amoebic ulceration.

Cultures from the left lung gave pure growths of this bacillus, whereas cultures from the dark red consolidated portions of the right lung gave negative results.

A culture from the spleen was negative.

The kidney patches gave a fairly copious growth of the bacillus.

Case 13. A Burman, 26 years old, admitted to hospital with a large gluteal abscess. Temperature upon admission 103.8° F.

Died upon the third day after admission.

P.M. An emaciated morphia injector with a large abscess in the left gluteal region (opened before death); the subcutaneous tissues of the thigh below the abscess were infiltrated with pus.

Lungs. Both lungs contain typical deposits of the new bacillary infection; more numerous in the left lung than in the right.

Spleen, slightly enlarged, with a few small nodules not very characteristic of the infection.

Cultures :

	Lung, pure growth of new bacillus.
Spleen, „ „	„ streptococci.
Thigh abscess, „ „	„ streptococci.

Case 14. Burmese, male, aged 30 years, admitted for tubercle of the lungs and diarrhoea; the history of the illness nil. There was impaired resonance at the right apex and moist rales all over; temperature 100° F. Bowels acted frequently; but no blood, only slime in the motions, which were offensive. Died two days after admission.

P.M. An ill-nourished morphia injector.

Pleura obliterated by well-organised adhesions.

Left lung, upper lobe normal, but in the lower lobe are a few small nodules of inflammatory consolidation.

Right lung, posterior portions engorged and oedematous, but no areas of consolidation.

Spleen, twice normal in size but no abscesses.

Large intestine, the mucous membrane largely destroyed by gangrenous ulcers of the amoebic dysentery type.

Case 15. Burman, aged 30 years, picked up in a dying condition in the streets : died soon after admission.

An emaciated morphia injector.

P.M. Lungs. Left lung, there are several small consolidated areas; one broken down into a cavity. The consolidation in appearance like that secondary to dysentery, and not like that due to an infection with this bacillus.

Right lung oedematous, otherwise normal.

Spleen, normal in size with well-organised adhesions around.

Heart, recent small vegetations on the mitral valves.

Large intestine covered with numerous large, gangrenous, amoebic dysenteric ulcers, which in the sigmoid are practically perforating.

Cultures. Lungs gave only a coliform organism.

Spleen, a pure growth of the bacillus under discussion.

Case 16. Burman, aged about 35 years, picked up in the street dead.

An emaciated morphia injector.

P.M. Lungs normal, but some old adhesions in the pleura.

A large amount of clear fluid in the abdominal cavity.

Liver shows well-marked multilobular cirrhosis.

Spleen, about four times normal in size, soft.

Large intestine covered with a large number of old, healed, and healing ulcers of the amoebic dysenteric type.

Smear from the spleen shows a few malaria parasites, and also a large amount of pigment.

A culture from the spleen gave half-a-dozen colonies of the new bacillus.

Case 17. A Burman, aged about 40 years, died in the out-patient department before examination: an emaciated morphia injector.

P.M. A few patches of the typical broncho-pneumonia were found in both lungs, particularly at their bases; there was a small fibrotic tuberculous induration at the right apex of the lung, otherwise the organs were apparently free from disease.

Pure growths of the bacilli were obtained from the consolidated areas of the lungs.

Case 18. A Hindu, aged about 30 years, picked up unconscious in the street, and died very shortly after being brought to hospital.

An emaciated morphia injector.

P.M. Posterior parts of both the upper and lower lobes of the left lung were extensively consolidated with what were then thought to be tubercular deposits: the right lung showed similar, but not quite such extensive, consolidation: the spleen was slightly enlarged.

Smears from the lungs showed non-acid-fast bacilli; cultures from the lungs, and from the spleen, gave copious growths of the bacilli in pure culture.

Case 19. A Burman, aged 33 years, admitted to hospital for general debility, and bronchitis, said to be of fifteen days' duration. The man was practically moribund upon admission, but when examined by a medical officer the suggestion was made that the infection was with this bacillus: unfortunately the patient died shortly after admission before any bacteriological examination could be made.

P.M. An ill-nourished body with numerous marks of morphia injection.

Lungs. Left lung, typical nodules on the lower lobe.

Right lung, numerous typical nodules scattered throughout.

At the apices of both lungs were old fibrotic patches, the remains of tubercular infection.

Spleen, twice the normal size with recent perisplenitis.

Other organs normal.

Cultures from both lungs and spleen gave copious pure growth of the bacilli.

Case 20. Burman, aged 40, admitted for fever, and cough of about twenty days' duration, in a weak, dying condition; there were signs of pneumonia upon the right side of the chest. A poorly nourished man with numerous marks of morphia injection; upon admission temperature 104° F., pulse 130, respirations 48. Died day after admission.

P.M. A moderately nourished Burman.

Lungs. Both lungs full of numerous typical nodules, the patches at the lower third of the upper lobe, right side, were coalescing so as to cause complete consolidation.

Spleen, slight enlargement, but no abscesses.

The lungs gave a pure luxuriant growth of the bacillus.

Case 21. A Burman picked up dead in 19th Street.

An emaciated morphia injector, aged about 28 years.

P.M. Lungs. Left upper lobe normal, lower lobe contains numerous patches of small areas of consolidation rather like miliary tubercular deposits.

Right lung, upper lobe one or two small suppurating cavities with a few greyish consolidated areas.

Middle lobe, oedematous, with large consolidated patches with purulent points.

Lower lobe, almost entirely consolidated with numerous very minute suppurating points.

Lung condition thought to be very likely tubercular.

Spleen, $1\frac{1}{2}$ times normal in size, fairly recent perisplenitis.

Liver, scarred, with well-organised adhesions to the diaphragm.

Large intestine, numerous recent and several old healed amoebic dysenteric ulcers.

Lung smear. No acid-fast bacilli, only basic staining bacilli.

Spleen smear, a few bacilli and cocci.

Cultures. From the lung a copious growth of the bacillus in pure culture.

From the spleen growths of coliform bacillus and a staphylococcus.

From the liver, coliform bacilli were grown.

Case 22. A Burman, aged about 33 years, died in the outdoor department before examination.

P.M. An emaciated body with very numerous marks of morphia injection.

Lungs. Left lung normal.

Right lung, upper lobe consolidated with what, in appearance, were acute tubercular deposits undergoing suppuration, the pus being thick and tenacious and of a greenish colour.

Middle lobe, a few deposits of acute broncho-pneumonia.

Lower lobe normal.

Spleen normal. Other organs normal.

Smears from the lung showed very numerous bacilli, but none acid-fast.

Cultures from the various diseased parts of the lung gave pure growths of the bacilli under consideration.

Case 23. A Hindu, male, aged about 48 years, picked up dead in the street. A moderately nourished old man with marks of morphia injection upon thighs and arms.

P.M. Lungs. Left lung, numerous coalescing patches of the infection with a small cavity at the apex, not tubercular in appearance.

In the middle and lower lobes a few discrete patches characteristic of the infection were found.

Spleen, slightly enlarged, with one or two tiny abscesses.

Other organs normal.

Smears from the lung patches showed bacilli to be present in large numbers.

In those from the spleen no bacilli were seen.

Cultures from the spleen gave a pure and vigorous growth of the bacillus; culture from the lung not taken.

Case 24. A well-nourished Hindu, male, aged about 28-30 years; dead body picked up in the street. Not a morphia injector.

P.M. Right lung, upper lobe in the lower half contains a large purulent cavity about the size of a tangerine orange; so far as appearances went this cavity was due to a breaking down of confluent patches of broncho-pneumonia the result of infection with this bacillus.

In the middle and lower lobes were very numerous patches typical of the infection.

Left lung, upper lobe middle portion, consolidated with confluent broncho-pneumonic patches of the infection.

Spleen, about twice the normal size, surrounded by numerous old, and recent, adhesions. Spleen substance soft. Other organs normal.

Smears and cultures from the lung gave numerous bacilli of the type under discussion.

Spleen culture not taken.

Urine cultivated but no growth of this bacillus obtained.

Case 25. A Mohammedan, male, aged 40 years, picked up dead in the street; an emaciated morphia injector.

P.M. Lungs. Left lung, upper lobe, numerous tubercular-like nodules, with a few more acute in appearance.

Lower lobe, a few similar small nodules.

Right lung, upper lobe, a big gangrenous foul-smelling cavity; with the rest of the lobe consolidated with what looked like small coalescing tubercular deposits. Other lobes normal. Other organs normal.

Smears from the lung showed the presence of a few acid-fast bacilli together with a fair number of non-acid-fast bacilli.

Cultures from the lung grew four colonies of the bacilli.

From the spleen a copious and pure growth of the bacillus was obtained.

Case 26. Burman, aged about 35 years, picked up dead in the street.

P.M. A moderately well-nourished body, with morphia injection marks upon both thighs.

Lungs. Left lung, a very few, tiny, nodules of characteristic appearance in the lower lobe; in the upper lobe was a little scarring, probably due to tubercular infection.

Right lung. Both upper and lower lobes full of characteristic nodules; while in the middle lobe were a few such nodules.

Other organs normal.

Cultures from the lungs and spleen gave copious and pure growths of the bacillus.

From the heart's blood a couple of colonies were obtained; while cultures from the groin and bronchial glands were sterile.

Case 27. Patient a Punjabi, male, aged 35 years.

Admitted to jail as a prisoner upon three years' sentence in September, 1910, in good general health; not an opium smoker or morphia injector.

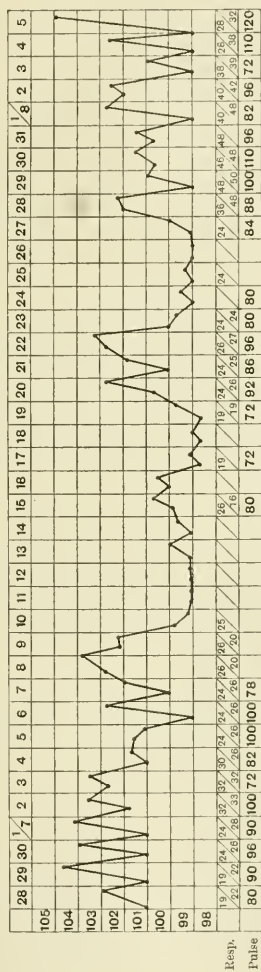
Admissions to hospital since coming to jail:

(1) February, 1911, for a bad attack of acne vulgaris.

(2) Suspicious symptoms of dysentery in February.

(3) Fever on the 16th of June.

Nothing of note in personal or family history.



Temperature chart of Case 27.

The prisoner admitted to the jail hospital upon the 28th of June and died August 7th.

Admitted to hospital for final illness on 28th June, complaining of fever.

Conditions upon admission : temperature 101.2° , pulse 80, resp. 19, a few moist rales in the left chest, and pleuritic pain.

June 29th, a single malarial parasite found in the blood.

July 2nd, sputum examined and tubercle bacilli not found.

July 3rd, swelling over the thyroid cartilage. Upon the 8th this swelling was incised and a little pus found. Friction rubs were audible over the right and left bases of the lungs. Upon the 10th a swelling formed over the right clavicle which was incised on the 11th. Upon the 14th swelling over the right trochanter, which was incised on the 18th. Upon the 20th began to have marked dyspnoea, and dulness was detected at the base of the right lung. Upon the 22nd there were signs of consolidation at the left base. Upon the 30th a swelling appeared over the left malleolus : incised on the 1st August. Sputum examined and neither pneumococci nor tubercle bacilli found.

The patient died unconscious upon the 7th August.

P.M. The abscesses had all been superficial, there was a small acute abscess on the surface of the left lobe of the liver; from this pus a film was made and stained with methylene blue and a few rod-shaped bacilli were seen.

With the exception of the lungs the other organs were healthy. Both lungs were the seat of an extensive but patchy consolidation, the larger patches presenting a whitish cheesy appearance. The smaller patches were reddish and surrounded by a zone of hyperaemia. From the lungs a pure culture of the bacillus under investigation was obtained without difficulty, and films showed these bacilli present in great numbers.

Case 28. Burman, male, age about 35 years; dead body picked up in the street. A well-nourished muscular man with a very few, apparently old, marks of morphia injections.

P.M. Lungs. Left lung, both lobes contain very numerous, but rather small, broncho-pneumonic nodules of about the size of a marble, fairly characteristic of the infection but a little more moist than usual in appearance.

Right lung, a few scattered nodules in the upper lobe; the other lobes normal.

Spleen, twice normal in size.

In the left kidney were three or four deposits the size of a two anna piece.

An abscess of the groin; and two abscesses over the right ankle.

Smears from the groin abscess showed staphylococci and streptococci, no bacilli.

From the ankle abscesses, a few possible bacilli.

Cultures from the lung, spleen, and kidney, gave colonies of the bacillus in pure growth.

Cultures from the ankle abscesses also grew the bacillus.

Case 29. A Mohammedan male, aged about 55 years, was admitted to hospital, suffering from cellulitis of the scrotum. He was in a very poor state of general nutrition; being a neglected morphia injector. Upon admission to hospital he was delirious, and obviously very ill: no history of his illness could be obtained. As he had not improved two days after efficient local treatment had been carried out, it was suspected that he was suffering from a septicaemia of which his scrotal condition was merely a symptom. When seen by a senior medical officer a suggestion

of this new infection was made, and the case reported to the laboratory for investigation.

He died three days after admission to hospital and although a full post-mortem examination was not carried out the lungs, liver and spleen were viewed. The lungs were found to be free from disease, but in both the liver and spleen were small inflammatory deposits in all respects similar to those, which had so frequently been seen in previous cases of this infection. Pure cultures of the bacilli were obtained from both the liver and spleen.

Case 30. A Burman, aged 38 years, admitted to hospital for general debility; duration of illness not stated.

A poorly nourished morphia injector, by trade a beggar; complains of cough with pain in the chest. Dulness was found over the apex and base of right lung, and rales and harsh breathing were heard over these areas: the clinical diagnosis was tubercular infection of the lungs. The sputum is described as frothy but not blood stained; no tubercle bacilli were detected upon examination. The patient had been in hospital for eight days when he died; during his stay in hospital his temperature was irregular, ranging from normal to 103° F.

P.M. There were numerous patches of acute broncho-pneumonia in both lungs; from these patches the bacilli were easily isolated in pure growth. Cultures from other organs were not attempted.

In addition to the disease of the lungs there were scars of old healed dysenteric ulcers in the large bowel, and a few, very small, open ulcers in the lowest few inches of the small bowel.

Blood serum from this case was taken, and its agglutinating power upon the bacilli isolated from other cases was tried; but no such power was found in any dilution of the serum.

Case 31. A Burman, about 40 years of age, admitted to hospital for general debility, and ulcers of both legs due to morphia injections; duration of illness said to have been of one month.

During his stay in hospital his temperature was irregular, ranging up to 101° F. He died after nine days in hospital.

P.M. An emaciated body.

Lungs. Both lungs contained very numerous nodules of acute broncho-pneumonia with the characteristic appearance of the infection.

Spleen of normal size, but covered with old, well-organised, adhesions.

In the lower portion of the large intestine were a few chronic ulcers.

The groin glands were fairly markedly enlarged.

Pure growths of the bacilli were obtained from the lungs and the spleen; but from the groin glands staphylococci only were isolated.

Case 32. Burman, male, picked up dead in the street, age about 28 years.

P.M. A poorly nourished body, with numerous marks of morphia injections.

Lungs. Left lung, upper portion of the lower lobe consolidated with coalescing patches of a typical appearance.

Right lung. Upper lobe practically solid, with similar patches, a few small scattered patches in the lower lobe.

Spleen $2\frac{1}{2}$ normal in size, with minute points of inflammatory deposits.

Liver contained very numerous, scattered, white points without any definite abscess formation.

Large intestine showed numerous scars of old dysenteric ulcers. Inguinal glands enlarged and upon the cut surface of the glands were areas of suppuration.

Cultures from the lungs and spleen gave a luxuriant growth of the bacillus.

From the liver and inguinal glands the bacilli were not isolated; but the inguinal glands gave a growth of staphylococcus.

Case 33. A poorly nourished Burman, of about 40 years of age, found in a dying condition in the street. Died shortly after admission to hospital. Duration of illness (fever and cough) said to have been two months.

A poorly nourished man with a few marks of morphia injection.

Numerous abscesses in the groin.

P.M. Lungs. Left, about a dozen small areas of broncho-pneumonic consolidation scattered throughout the lung.

Right lung. In upper and middle lobes, about five or six small nodules present.

Lower lobe almost completely solid with confluent patches of a typical appearance.

Spleen of normal size; a little recent deposit of inflammatory lymph on the surface.

Kidneys. Both kidneys contain five or six fairly typical small and large patches of this infection.

Smears from the lung and kidney show very numerous bacilli.

Culture from the spleen grew a pure growth (about half-a-dozen colonies) of the bacilli.

From the groin abscesses the bacillus was not isolated.

Case 34. Burman, aged 21 years, admitted to hospital with the symptoms of acute dysentery; which was the clinical diagnosis. The man died ten days after admission: his temperature during his stay in hospital had been normal until the last day.

P.M. An emaciated body with numerous marks of morphia injections.

Lungs. Both lungs contained very numerous deposits of a typical appearance.

The large bowel was extensively ulcerated, the ulcers being those of the ordinary amoebic dysentery.

Cultures from the lungs alone were taken and gave luxuriant growths of the bacilli.

Case 35. A Hindu, male, aged about 33 years, picked up in a dying condition in the street.

P.M. A well-nourished man, no evidence of morphia injections present.

Lungs. Right lung, normal.

Left lung, acute pleurisy over the lower half of the lung, with a few haemorrhagic patches of consolidation in the lower lobe, more like those due to a terminal dysenteric infection than to the infection under discussion; but in the upper lobe was one patch with the characteristics of this infection.

Large bowel in a condition of acute dysenteric ulceration.

Cultures from the haemorrhagic patches in the lower lobe gave growths of a coliform organism; while a culture from the patch of different appearance in the upper

lobe gave a pure growth of the new bacillus. This culture was proved, both by sub-culture, and by animal inoculation.

Case 36. A Chinaman, about 27 years old, admitted to hospital for general debility, and diarrhoea, of one month's duration.

Died after seventeen days' stay in hospital; during this time he was suffering from diarrhoea, general loss of appetite, and rapid wasting. His temperature was irregular and he had a cough, of which no very serious notice was taken; his lung condition was attributed to the terminal broncho-pneumonia not uncommon in wasted dysenteries; his history was unknown to us, as he spoke only an incomprehensible Chinese dialect, and had no friends. He was not a morphia injector.

P.M. An emaciated body.

Lungs. Left lung was full of very numerous nodules of acute broncho-pneumonia of a very characteristic appearance.

The right lung was free from any obvious disease.

The spleen was normal in appearance.

In the large intestine were fairly numerous, very tiny, acute, shallow ulcers, such are not uncommon in diarrhoea cases apart from true dysentery.

Cultures were taken from the lung and spleen and from both sources pure growths of the bacillus were obtained.

Case 37. Hindu, male, aged about 22 years; the dead body was picked up in the street.

P.M. An emaciated body, with numerous marks of morphia injections.

Lungs. Several small nodules scattered throughout both lungs, more numerous in the left than in the right lung, of a character typical of the infection.

Spleen, normal in size, but soft.

Intestine showed chronic inflammation of the bacillary dysentery type, both small and large intestines being infected.

Other organs were normal.

Cultures from the lung nodules gave pure and vigorous growths of the bacillus.

Those from the spleen gave only a few colonies.

Case 38. A Burman, about 25 years of age, admitted for looseness of bowels with pain in the abdomen and back, of about four months' duration.

Temperature irregular, ranging from 99° F. to 102° F.: the patient remained in hospital for eighteen days; then died.

P.M. An emaciated Burman with a few marks of morphia injections.

Lungs. Both lungs contain numerous typical nodules of the infection.

Peritoneum, acute peritonitis present.

Large intestine covered with large gangrenous ulcers of amoebic dysentery, which have penetrated to the peritoneal coat, and caused the peritonitis. Amoebae present in the ulcers.

Spleen, normal in appearance.

Cultures from the lung nodules gave very vigorous growths of the bacilli.

From the spleen a fair number of colonies of the bacillus grew.

DESCRIPTION OF PLATE I.

Fig. 1. Section of the lung of Case I, showing single and confluent areas of consolidation.

This illustration was painted by a Native artist, quite ignorant of the particular points which the specimen intended to represent; therefore, though these points may not be as plain as could be wished, yet the painting has the merit of representing the specimen as seen by an unbiassed observer.

Fig. 2. Agar culture, one month old.

Fig. 3. Agar culture, cultivated for 48 hours.

THE INHIBITORY SELECTIVE ACTION ON BACTERIA OF BODIES RELATED TO MONO-CHLORACETIC ACID.

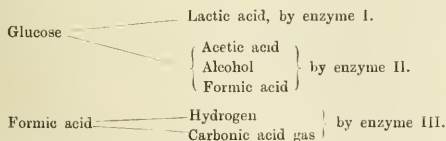
(A CONTRIBUTION TO THE THEORY OF CELL INTOXICATION.)

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(With Plate II.)

THE author (1911) showed that growth of *B. coli* (Escherich), on monochloracetic acid agar, led to the selection of a new strain unable to produce gas from glucose, lactose and other sugars, but still able to produce gas from alcohols in very considerable amount. Harden and Penfold (1912) compared the chemical action of this new strain with that of the original strain when grown anaerobically on glucose peptone water. The general result of the examination was to show that the new strain produced much more lactic acid, and less alcohol, acetic acid and formic acid, the gas being reckoned as formic acid, than the original strain. This pointed to the fact that the lactic acid-producing ferment was distinct from that ferment which produced alcohol, acetic acid and formic acid. Harden (1901) had previously brought forward evidence showing that the CO_2 and H were derived from the splitting up of formic acid. Since the new strain which could not produce gas from glucose, produced a normal yield from sodium formate, Harden and Penfold concluded that the splitting up of the formate was due to a third enzyme. This fermentation of glucose is shown schematically below:



Six different strains of coliform organisms, and also *B. enteritidis* Gaertner, *B. paratyphosus* B. and *B. Grünthal*, were found constantly to give non-gas-producing strains when grown on chloracetate agar and tested in glucose. It was no accidental variation, for the new strains could be produced with certainty in ten days.

In the endeavour to understand the above selection process, various organic substances were added to agar and the evidences of variation

TABLE I.

The cultural signs of variation and selection obtained when B. coli (Escherich) is grown on phenylacetic acid agar plates.

No. of plate	Quantity of 10 ² / ₁₀ solution of phenylacetic acid added to respective plates	Days of observation		
		2nd	4th	7th
1	None	Good growth. No variability in the size of the colonies. No secondary colonies present.	No secondary colonies. No variability in the size of the colonies other than that due to crowding.	As last observed.
2	None	"	"	"
3	0.05 c.c.	"	Definite naked eye papillae present on the colonies.	Many large papillae present on colonies. Marked variability in size of colonies.
4	0.3 c.c.	"	"	"
5	0.5 c.c.	"	"	"
6	0.7 c.c.	"	Some colonies show swollen margins.	"
7	1.0 c.c.	"	Papillae present on some colonies. Big and little colonies are present in central crowded portions of plate.	"
8	1.3 c.c.	"	"	"
9	1.6 c.c.	"	"	"
10	2.0 c.c.	"	"	"
11	2.4 c.c.	Colonies inhibited in growth a little.	"	"
12	2.8 c.c.	Colonies inhibited in growth markedly.	As last observed.	"

Remark. In the higher concentrations many of the colonies of large size are thickened and of a marked yellow colour.

and selection which had been obtained with chloracetate sought for. Some of these results are detailed in previous papers by the author.

It was found *e.g.* that monochloracetate of soda was much more inhibitory than dichloracetate or trichloracetate. Neither the dichloracetate nor trichloracetate produced the papillae on the surface of the plate colonies which are so characteristic of the monochloracetate. Great variability in the size of the colonies—a feature which was strongly marked in the case of the monochloracetate plate cultures—did not occur.

TABLE II.

B. coli after growth on phenylacetic acid agar.

Agar slopes, from colonies of phenylacetic acid agar plates described in Table I, were inoculated in glucose and mannite peptone waters with the following result.

From plate 10 of the series.

Variety of colony from which the agar slope was taken	Day of observation	Glucose	Mannite
(1) Large yellow colony	1	Af. G. 1/6	Af. G. 4/10
	2	Af. G. 1/5	Af. G. 5/6
(2) „	1	Af. G. 1/3	Af. G. 4/10
	2	Af. G. 5/12	Af. G. 2/3
(3) „	1	Af. G. 1/5	Af. G. 1/4
	2	Af. G. 5/12	Af. G. 3/4
(4) „	1	Af. G. 1/3	Af. G. 1/2
	2	Af. G. 5/12	Af. G. 5/6
(5) „	1	Af. G. 1/6	Af. G. 1/3
	2	Af. G. 1/3	Af. G. 2/3
(6) Little colony	1	Af. G. 1/6	Af. G. 1/2
	2	Af. G. 1/3	Af. G. 2/3
(7) „	1	Af. G. 1/4	Af. G. 7/16
	2	Af. G. 1/3	Af. G. 2/3
(8) „	1	Af. G. 1/4	Af. G. 7/12
	2	Af. G. 1/3	Af. G. 2/3

From plate 11 of the series.

(A) Large colony	1	Af. G. 1/3	Af. G. 7/12
	2	Af. G. 5/12	Af. G. 7/12
(B) „	1	Af. G. 1/5	A. 3/4 G. 7/16
	2	Af. G. 1/3	Af. G. 5/6
(C) „	1	Af. G. 1/4	Af. G. 1/3
	2	Af. G. 1/3	Af. G. 2/3
(D) „	1	Af. G. 1/4	Af. G. 1/5
	2	Af. G. 1/3	Af. G. 1/2
(E) „	1	Af. G. 3/8	Af. G. 1/3
	2	Af. G. 1/2	Af. G. 3/4

From plate 12 of the series.

(F) Large yellow colony	1	Af. G. 1/3	Af. G. 1/2
	2	Af. G. 5/12	Af. G. 2/3
(G) „	1	Af. G. 3/8	Af. G. 1/2
	2	Af. G. 5/12	Af. G. 3/4
(H) „	1	Af. G. 1/2	Af. G. 7/16
	2	Af. G. 7/12	Af. G. 3/4

Monobromacetate of soda added to agar gave the same papillae as the monochloracetate but big colonies did not appear in high concentrations as in the case of monochloracetate plates, and from none of the plates with lower concentrations, was it possible to obtain non-gas-producing strains.

Phenylacetic acid. Phenylacetic acid was dissolved in saturated sodium carbonate solution until the solution was just faintly alkaline to neutral litmus paper. Distilled water was then added to render the solution of 10% strength. The solution was sterilized by filtration through a Doulton filter. Ascending quantities of the solution were put into Petri dishes and 15 c.c. of melted agar were added to each plate, the whole being then well mixed.

The plates were inoculated with *B. coli* (Escherich) and grown at 37° C. Their appearances are indicated in Table I.

The results recorded in Table I are similar to those obtained when *B. coli* (Escherich) was grown on chloracetic acid agar.

From plates 10, 11 and 12 of the series, after eight days' growth at 37° C., various colonies were inoculated on to agar slopes and subsequently tested on mannite and glucose peptone waters. The results are recorded in Table II¹.

It will be noticed that the average yield of gas of the strains from big colonies of plate 10 was the same as that from the little colonies, so that the phenylacetic acid does not appear to differentiate between the colonies in respect of gas-forming power. The gas-producing function in all the colonies is, however, slightly depressed.

Other substances which were tried, were found ineffectual in producing marked selections on the plates or non-gas-producing variants, but details of the experiments are omitted here because they are elsewhere dealt with and because of the negative results. The substances in question were cyanoacetic acid, α -bromopropionic acid, dibromsuccinic acid, chlormalonic acid, hippuric and benzoic acid, all used as alkaline salts. On the other hand monochlorhydrin, a monochlor substitution product of glycerine, was found to give rise to a similar variant in the case of the *B. coli*, as monochloracetate of soda. The appearances indicating selection as they occurred in the colonies of the monochlorhydrin agar plates were somewhat different from those observed on monochloracetate of soda plates.

¹ In Table II Af.=full acid reaction. G.=gas. The fraction after G.=the amount of the gas tube occupied with gas. The gas tests were performed in Durham's tubes. The fractions following A. indicate varying degrees of partial acidity, of the litmus solutions.

The *monochlorhydrin* medium is an agar to which a known quantity of 20% filtered solution of monochlorhydrin is added. Ascending quantities of the solution were placed in Petri dishes and 15 c.c. of melted agar were added to each plate and thoroughly mixed. The plates were then inoculated with *B. coli* and grown at 37° C. The appearances obtained are given in Table III.

Table III shows, therefore, that at suitable concentrations marked variability in the size of similarly situated colonies may occur, and papillae may be present, which however are more inclined to be situated towards the cortex of the colonies than in the case of colonies of *B. coli* when grown on monochloracetate agar. The most peculiar feature of the plates is the large tree-like outgrowths from the periphery of the colonies. These seem to have some similarity to the appearances described by R. Müller (1909) in colonies of *B. paratyphosus* B. when grown on gelatine. (Pl. II, figs. 1, 2, 3.)

Subcultures on to agar slopes were made from different colonies of the series described in Table III and were then tested on carbohydrate media as recorded in Table IV.

The results of the examination of the different colonies and portions thereof show that the dense colonies and the papillae are good gas producers, whereas on chloracetate of soda plates the big dense colonies were either poor gas producers or failed to produce gas at all.

The lateral tree-like expansions of the colonies on the 0.2 c.c. chlorhydrin plates had, however, lost the power of producing gas from lactose. Two of these were tested on the whole series of carbohydrates with results given in Table V.

Table V shows that the variant *B. coli* obtained by growth on chlorhydrin agar is similar to the monochloracetate variety of the same organism. It gives either little or no gas from sugars and a fair yield from alcohols and it takes a longer time than normal to clot milk. On the other hand it appears almost entirely unable to produce gas from sodium formate while the normal strain gives 6/12 to 7/12 of a tubeful as also did the chloracetate variety of *B. coli* which I first described. It would be very interesting to know if an accumulation of formic acid is taking place in the glucose tubes on which this new variety is grown, but that point has not yet been decided.

TABLE III.

Record of plates of *B. coli* (*Escherich*) on monochlorhydrin agar. Each plate contained 15 c.c. of agar and the quantity of 20% chlorhydrin solution indicated.

Amount of chlorhydrin solution	Day of observation			
	1st	2nd	5th	10th
None	Good growth.	Edges of colonies somewhat spreading in character.	As last observed.	No lateral expansions, no papillae, no variability in size of the colonies in centre of plate.
.1 c.c.	Slight inhibition of growth.	Edges of colonies sharper, but the colonies are well grown.	Lateral expansions present on all large colonies. Cortical papillae also present on colonies.	A few small opaque dense colonies outgrowing the rest in the crowded centre of plate. (See Pl. II, fig. 1.)
.2 c.c.			"	The lateral expansions of these colonies have a tree-like appearance and are better developed than in any other concentration. Many papillae are present on the colonies, and dense colonies are present on the centre of the crowded plate. (See Pl. II, fig. 2.)
.35 c.c.			"	The expansions are however smaller and not so tree-like.
.5 c.c.			Marginal colonies of large size show swollen edges. Big and little colonies are present in the centre of the plate.	Many papillae present on the colonies.
1.0 c.c.			The colonies on this plate show marked inhibition but are of uniform size.	As observed 5th day. (See Pl. II, fig. 3.)
1.5 c.c.	Marked inhibition.	As observed 1st day.	A few of the colonies in crowded part of plate are of a larger size than the rest.	
2.0 c.c.	Sterile.	Sterile.	Sterile.	

All observations are naked eye unless otherwise stated.

TABLE IV.

The plate in the series from which the subculture was made	Type of colony or portion of colony from which the sub- culture was made	Days of obser- vation	Lactose	Glycerine	Saccharose	Broth	Indol	Motility and staining
0.1 c.c. plate	Lateral expansion of colony.	1	Af. G. 1/2	An. Gn.	An. Gn.	Cloud	++	+
		2	Af. G. 7/12	"	"			Gram
		6	Af. G. 1/2	A. 1/2 G. 1/6	"			negative
	"	1	Af. G. 1/2	An. Gn.	"	Cloud	++	+
		2	Af. G. 3/5	"	"			Gram
		6	Af. G. 7/12	A. 1/2 G. 1/12	"			negative
	Dense colony of crowded portion of the plate.	1	Af. G. 1/8	An. Gn.	"	Cloud	++	+
		2	Af. G. 5/12	"	"			Gram
		6	Af. G. 5/12	Af. G. 1/2	"			negative
0.2 c.c. plate	"	1	Af. G. 1/3	An. Gn.	"	Cloud	++	+
		2	Af. G. 5/12	"	"			Gram
		6	Af. G. 7/12	A. 1/2 G. 1/6	"			negative
	Lateral expansion of colony.	1	Af. Gn.	An. Gn.	"	Cloud	++	+
		2	"	"	"			Gram
		6	"	A. 1/2 G. 1/6	"			negative
	"	1	"	An. Gn.	"	Cloud	++	+
		2	"	As. Gn.	"			Gram
		6	"	A. 1/2 G. 1/8	"			negative
0.5 c.c. plate	"	1	Af. G. 7/12	An. Gn.	"	Cloud	++	+
		2	Af. G. 7/12	"	"			Gram
		6	Af. G. 1/12	"	"			negative
	Papillae.	1	Af. G. 5/12	"	"	Cloud	++	+
		2	Af. G. 1/2	"	"			Gram
		6	Af. G. 1/2	An. G. 1/6	"			negative
	"	1	Af. G. 5/12	An. Gn.	"	Cloud	++	+
		2	Af. G. 1/2	"	"			Gram
		6	Af. G. 1/2	A. 1/2 G. 1/12	"			negative
	Dense colony of crowded portion of the plate.	1	Af. G. 5/12	An. Gn.	"	Cloud	++	+
		2	Af. G. 1/2	"	"			Gram
		6	Af. G. 5/12	A. 1/2 G. 1/10	"			negative
	"	1	Af. G. 1/2	An. Gn.	"	Cloud	++	+
		2	Af. G. 7/12	"	"			Gram
		6	Af. G. 1/2	Af. G. 1/2	"			negative
	"	1	Af. G. 1/2	An. Gn.	"	Cloud	++	+
		2	Af. G. 1/2	"	"			Gram
		6	Af. G. 1/2	A. 1/2 G. 5/12	"			negative

An.=no acid. Gn.=no gas. As.=slight acid reaction.

TABLE V.

Strain	Day of observation	Arabinose	Xylose	Isodulcitol	Glucose	Lactulose	Mannose	Gulactose	Maltose	Lactose
(1) Lateral expansion of a colony of a 0-2 c.c. chlorhydrin plate.	1	Af. Gn.	Af. Gn.	Af. G. bub.	Af. Gn.	Af. Gn.	Af. G. bub.	Af. Gn.	Af. Gn.	Af. Gn.
	2	"	Af. G. bub.	"	"	"	"	"	Af. G. bub.	"
	6	Af. G. bub.	"	"	"	"	"	"	Af. G. 1/4	"
(2) Second lateral expansion of a colony of the same plate.	1	Af. Gn.	Af. Gn.	Af. Gn.	"	"	"	"	Af. Gn.	An. Gn.
	2	Af. G. bub.	Af. G. bub.	Af. G. bub.	"	"	"	"	"	"
	6	Af. G. bub.	"	"	"	"	"	"	"	"
(3) Control of normal <i>B. coli</i> (Escherich).	1	Af. G. 5/12	Af. G. 5/12	Af. Gn.	Af. G. ?	Af. G. 5/12	Af. G. 5/12	Af. G. 1/3	Af. G. 5/12	Af. G. 7/12
	2	Af. G. 1/2	Af. G. 7/12	"	Af. G. 1/2	Af. G. 1/2	Af. G. 1/2	Af. G. 1/2	Af. G. 7/12	Af. G. 3/4
Cane sugar										
(1) Lateral expansion of a colony of a 0-2 c.c. chlorhydrin plate.	1	An. Gn.	An. Gn.	Dextrin	Inulin	Sulcin	Amorphin	Glycerine	Erythrit	Adonite
	2	"	"	As. Gn.	An. Gn.	An. Gn.	Green, no gas	An. Gn.	An. Gn.	-
	6	"	"	A. 1/2 Gn.	"	"	"	A. 3/4 G. 1/2	"	"
(2) Second lateral expansion of a colony of the same plate.	1	"	"	As. Gn.	"	"	"	An. Gn.	"	"
	2	"	"	A. 1/2 Gn.	"	Af. Gn.	"	Af. G. 1/4	"	"
	6	"	"	"	"	"	"	"	"	"
(3) Control of normal <i>B. coli</i> (Escherich).	1	"	-	Gp. An.	-	-	-	A. ? G. 1/12	-	-
	2	"	-	Gp. A.	-	-	-	-	-	-
Sorbit										
(1) Lateral expansion of a colony of a 0-2 c.c. chlorhydrin plate.	1	Af. Gn.	Af. G. 5/12	Dulcitol	Sodium formate	Milk	Broth	Modifiy and staining	Indol	
	2	Af. G. 1/3	Af. G. 5/12	An. Gn.	An. Gn.	A. No clot	Cloud	+ Gram negative		
	6	Af. G. 5/12	Af. G. 5/12	Af. G. 5/12	An. G. bub.	A. Clot	"	"		
(2) Second lateral expansion of a colony of the same plate.	1	Af. Gn.	Af. G. 5/12	An. Gn.	An. Gn.	A. No clot	"	+		
	2	Af. G. 1/3	Af. G. 5/12	Af. G. 5/12	An. G. bub.	"	"	Gram negative		
	6	Af. G. 5/12	Af. G. 5/12	Af. G. 5/12	An. G. bub.	A. Clot	"	"		
(3) Control of normal <i>B. coli</i> (Escherich).	1	Af. G. 5/12	Af. G. 7/12	A. 1/2 G. bub.	G. 1/2	A.	"	+		
	2	Af. G. 7/12	Af. G. 3/4	Af. G. 7/12	G. 7/12	A. Clot	"	Gram negative		

B. lactis aerogenes.

We will now consider the response of *B. lactis aerogenes* to certain selecting agents.

Several experiments made with the object of selecting out non-gas-producing varieties of *B. lactis aerogenes* on monochloracetate agar proved unsuccessful and it was considered probable that the non-success was due to the very different fermentation of sugar effected by this organism as compared with *B. coli* (Escherich).

Attempts were however made with chlorhydrin agar to obtain variants of *B. lactis aerogenes* and I would like to submit here a preliminary notice of the results.

Appearance of B. lactis aerogenes when grown at 37° C. on chlorhydrin agar plates.

Plates containing 1 c.c. of 20% chlorhydrin solution to 15 c.c. of agar gave fairly good growth by the second day though not so good as controls without chlorhydrin, and from the second day onward, in the crowded portions of the plates, a few colonies could be seen which were denser and more opaque than their neighbours. The difference however was slight, and not at all so marked as that shown by colonies of *B. coli* (Escherich) on plates of the same strength. After ten days' growth two colonies which projected slightly from the centre of the plate were inoculated on to agar slopes. These slopes grew slowly at 37° C.—probably because some of the chlorhydrin may have been carried over with the organisms. On the third day, however, the agar slopes were well covered with a thick growth. These cultures were then tested on a small series of carbohydrate media when one of them (Strain "6") was found to have lost the power to ferment glycerine.

The other strain retained that power in normal amount. Strain "6" was then plated out and two separate colonies tested again. They each gave the same results as before in respect of glycerine-fermentation. The loss of fermentation power was confined to glycerine, while mannite and glucose gave good yields of gas.

Strain "6" was finally tested on a large series of carbohydrate media along with a control of the normal strain and gave the results shown in Table VI.

A consideration of Table VI shows us that the chlorhydrin variety of *B. lactis aerogenes* gave acid and gas in the same sugars and alcohols

Inhibition of Bacterial Growth

TABLE VI.

Strain	Day of observation	Aralbinose	Isodulcitol	Glucose	Laevulose	Mannose	Galactose	Maltose	Lactose	Cane sugar
<i>B. lactis aerogenes</i>	2	As. G. 1/12	Af. G. 1/10	Af. G. 7/12	Af. G. 1/2	Af. G. 5/12	Af. G. 1/4	Af. G. 1/7	Af. G. 7/16	Af. G. 5/8
Strain 6,	6	As. G. 1/12	Af. G. 1/10	Af. G. 1/2	Af. G. 1/2	Af. G. 5/12	Af. G. 1/4	Af. G. 1/7	Af. G. 1/2	Af. G. 1/2
Normal <i>B. lactis</i>	2	As. G. 1/8	Af. G. 1/8	Af. G. 2/3	Af. G. 7/10	Af. G. 1/2	Af. G. 1/2	Af. G. 4/5	Af. G. 9/10	Af. G. 2/3
<i>aerogenes</i> .	6	As. G. 1/8	Af. G. 1/12	Af. G. 1/2	A. 1/2 G. 1/3	Af. G. 1/3	Af. G. 1/3	Af. G. 5/8	Af. G. 1/2	Af. G. 1/2
<i>B. lactis aerogenes</i>	2	Raffinose	Dextrin	Inulin	Salicin	Amygdalin	Glycerine	Erythritol	Adonite	Sorbitol
Strain 6,	6	Af. G. 3/4	Af. G. 1/8	-	Af. G. 1/3	-	-	-	Af. G. 3/4	Af. G. 1/12
Normal <i>B. lactis</i>	2	Af. G. 1/2	Af. G. 1/6	-	Af. G. 1/4	-	-	-	Af. G. 2/3	Af. G. 1/2
<i>aerogenes</i> .	6	Af. G. 5/8	Af. G. 5/8	-	Af. G. 3/8	-	Af. G. 5/8	-	Af. G. 7/16	Af. G. 1/2
		A. 1/2 G. 1/3	Af. G. 1/8	-	Af. G. 1/4	-	A. 1/2 G. 1/3	-	Af. G. 5/16	Af. G. 1/2
<i>B. lactis aerogenes</i>	2	Mannitol	Dulcitol	Sodium formate	Milk	Broth	Motility	Indol	V. & P.	
Strain 6,	6	Af. G. 11/12	-	-	Af.	Cloud	Non-motile	-	+	
Normal <i>B. lactis</i>	2	Af. G. 4/5	-	-	A. Clot	-	-	-	-	
<i>aerogenes</i> .	6	Af. Gf.	-	-	"	Cloud	Non-motile	-	+	
		Af. G. 3/4	-	-	"	-	-	-	-	

as the normal strains except in the case of glycerine. The new variety appeared to have lost a single ferment and that particular ferment was probably closely related chemically to the toxic agent used in the selection process.

The whole process was repeated and a strain (No. 13) obtained which gave the same result.

The process was again repeated with the results given in Table VII from which we see that strain "F" gave only slight fermentation of glycerine by the sixth day, and that strain "G" gave no acid or gas by the fourth day but on the sixth day showed acid without gas.

All these altered strains of *B. lactis aerogenes* reacted with Voges and Proskauer's test and were non-motile and did not liquefy gelatine.

TABLE VII.

Strain	Day of observation	Glucose	Mannite	Adonite	Glycerine
0.5 c.c. <i>B. lactis aerogenes</i> (large) A.	2	Af. G. 12/12	Af. G. 7/8	Af. G. 1/20	Af. G. 1/2
	4	A. 1/2 G. 7/12	Af. G. 9/10	Af. G. 1/10	Avs. G. 1/2
	6	A. 1/4 G. 7/12	Af. G. 4/5	Af. G. 1/9	Avs. G. 4/10
0.5 c.c. (large) B.	2	Af. G. 1/6	Af. G. 11/12	Af. G. 1/4	Af. G. 1/2
	4	A. 1/2 G. 1/2	Af. G. 7/8	Af. G. 3/10	Avs. G. 7/16
	6	A. 3/4 G. 5/12	Af. G. 4/5	Af. G. 1/4	Avs. G. 4/10
0.5 c.c. (large) C.	2	Af. G. 9/10	Af. G. 11/12	Af. G. 5/12	A. 3/4 G. 7/12
	4	Af. G. 9/16	Af. G. 9/10	Af. G. 5/12	Avs. G. 1/2
	6	A. 1/2 G. 1/2	Af. G. 5/6	Af. G. 2/5	Avs. G. 4/10
0.5 c.c. Lat. Exp. D.	2	Af. G. 12/12	Af. G. 12/12	Af. G. 1/6	Af. G. 5/12
	4	A. 3/4 G. 5/8	Af. G. 12/12	Af. G. 1/5	A. 1/2 G. 1/2
	6	A. 1/2 G. 5/8	A. 3/4 G. 11/12	Af. G. 1/6	Avs. G. 4/10
0.5 c.c. Lat. Exp. E.	2	Af. G. 7/8	Af. G. 12/12	Af. G. 1/5	A. 1/4 G. 7/12
	4	A. 1/2 G. 5/12	Af. G. 9/10	A. 1/4 G. 1/5	A. 1/4 G. 1/2
	6	A. 1/2 G. 7/12	Af. G. 5/6	Af. G. 1/5	Avs. G. 5/12
0.5 c.c. Lat. Exp. F.	2	Af. G. 3/4	Af. G. 11/12	Af. G. 5/12	—
	4	A. 3/4 G. 1/2	Af. G. 11/12	Af. G. 5/12	—
	6	A. 3/4 G. 1/2	Af. G. 7/8	Af. G. 4/10	A. 1/2 G. 1/16
1.5 c.c. Big col. G.	2	Af. G. 1/4	Af. G. 4/5	Af. G. 1/3	—
	4	Af. G. 3/8	Af. G. 11/12	Af. G. 4/10	—
	6	Af. G. 1/3	Af. G. 5/6	Af. G. 3/8	Af. Gn.
1.5 c.c. Big col. H.	2	Af. G. 1/2	Af. G. 4/5	Af. G. 4/10	A. 1/2 G. 7/16
	4	A. 3/4 G. 2/3	Af. G. 12/14	Af. G. 3/8	A. 1/4 G. 1/2
	6	A. 3/4 G. 1/2	Af. G. 7/8	Af. G. 1/2	A. 1/16 G. 1/2

Avs. = very slight reaction.

After being grown on ordinary agar for two months, strains "13" and "G" were tested again on glycerine peptone water. "13" gave 1/8 of a tubeful of gas by the sixth day; the other strain gave no gas whatever after six days' growth, though it gave a late acid reaction.

I have tested on glycerine peptone water over 50 colonies of the strain of *B. lactis aerogenes* used, and have not yet met one which failed to give a good yield of gas in one day.

Theoretical considerations.

The loss of the glycerine-fermenting power of *B. lactis aerogenes* when grown in the presence of a toxic substitution product of glycerine suggests that the selection might be due to intoxication of the glycerine-fermenting bacteria by the agency of the glycerine ferment. It seems on the face of it more than a coincidence that chlorhydrin permits the selection of a non-glycerine-fermenting strain. The loss of the gas-forming power of *B. coli* when grown on monochloracetate agar was thought possibly to be due to intoxication, by the agency of the formic acid-producing enzyme, of those bacteria having a relative excess of this enzyme. This enzyme produces also acetic acid and probably therefore has a chemical affinity with acetic acid and hence with chloracetic acid. Harden has shown that the formic acid is the source of the gas, so that any toxic agent removing the formic acid-producing ferment would deprive the organism of the source of gas¹. This explanation appears, however, doubtful, in face of the fact that chlorhydrin produces a very similar though not identical variety of *B. coli*. The intoxication and selection may be due to the CH_2Cl group common to each compound, and the acetate structure, as such, may have no specific effect.

The portion of the cell reacting with this group is obscure as there seems no reason to believe that the formic acid-producing ferment has a special affinity with it. Chloracetic acid has not been able in my experience to remove the glycerine-fermenting power of *B. lactis aerogenes*, so that we appear to have some more specific intoxication in the case of this organism with this substance.

¹ Harden and Walpole (1905) showed that the percentage yield of acetic acid in the decomposition of glucose by *B. lactis aerogenes* was only about one-third of that obtained when *B. coli* (Escherich) fermented this sugar. Now if the acetic acid producing enzyme were the agent of intoxication one would expect chloracetate to be much less effective in the case of *B. lactis aerogenes* than in that of *B. coli* (Escherich). This appears to be the case.

The possibility of ferment intoxication led me to try lactose phenylhydrazone as a selecting agent hoping therewith to obtain a non-lactose-fermenting variant. It was soon evident that the intoxication of the bacteria was conditioned by the NH group in this substance. The hydrogen was therefore replaced by a methyl group when the toxicity of the substance was found to be greatly reduced. This methyl derivative is being used at present but the work is not sufficiently advanced to permit of any statement of its effects as a selecting agent.

Ehrlich has held for some time that the nutriceptors play a part in cell intoxication. To prove this, one ought to be able to show that the surviving cells, after a selective intoxication with the given agent, are not able to use a definite food which the original cells used, or at least do not use it to the same extent. This view does not appear as yet to rest on any experimental basis¹.

SUMMARY.

1. *B. coli* (Escherich) produces papillated colonies and shows marked variability in the size of its colonies when it is grown on agar to which phenylacetic acid has been added in the form of the sodium salt.

The big and little colonies produce about the same amount of gas when tested on glucose.

2. When *B. coli* (Escherich) is grown on agar containing monochlorhydrin it throws off variants similar to those produced when grown on monochloracetate of soda media. Speaking broadly they ferment alcohols with gas formation, and sugars without gas formation.

3. *B. lactis aerogenes* grown on monochlorhydrin agar gives rise to variants unable to ferment glycerine.

4. In cases of inhibitory bacterial selection by chemical agents a careful comparison of the surviving cells with the original strain from which they were derived is calculated to indicate that portion or function of the cell which is implicated in the cell's intoxication. This question does not seem to have been attacked hitherto from this standpoint.

5. The cell ferments by virtue of their specific chemical affinities may play a part in cell intoxication.

¹ It appears probable that the cell enzymes frequently play a secondary part in cell intoxication. We know for example that carbolic acid is rendered much more germicidal by the addition of acids. Now in many media the cell enzymes will produce acids, hence it is probable that carbolic acid selections of bacteria commonly result in the development of new strains with impaired fermenting power.

I desire to express my indebtedness to the authorities of the Lister Institute and to the "Constance Research Fund" administered by Dr E. C. Hort for all the experimental facilities which made the work of this paper possible.

My thanks are likewise due to Dr Ledingham for valuable help and criticism during the course of this research.

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Note: For description of Plate II, see Table III, p. 40.

NOTES ON SOME TROPICAL DISEASES OF PALESTINE.

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(With 1 Chart.)

Introduction.

JERUSALEM is the resort of so many tourists and pilgrims who chiefly visit the country in the healthy spring season and see little of the conditions in which the poor of the land live, that it may be difficult for them to realize that it is a city where malaria is exceedingly rife and where the most elementary sanitary regulations are utterly neglected. There is no proper public water supply, no sewers worth the name, vaccination is not enforced even when small-pox is epidemic and there is no attempt to isolate those suffering from infectious diseases. This state of things is undoubtedly largely due to remissness and ignorance on the part of the Turkish Government, but the situation is made very complicated and difficult on account of the "Capitulations" by which all foreign subjects are practically independent of the Local Authority and accept no orders except through their own Consuls. It is easy to see that any government in the world would find it exceedingly difficult to enforce measures of public health—which are often in our own lands obstructed as far as possible by lay persons—under circumstances like these. The inhabitants of a single Jewish tenement-house may be representatives of perhaps half a dozen nations and not one step can be taken by the Local Authorities to get rid of any public nuisance until the assent of the Consuls of all six "powers" concerned is obtained. To those who have had experience of human nature, it will not be surprising to learn that unanimity in such a case has so far been almost impossible to obtain. Undoubtedly one of the reasons has been that

hitherto there has been no authority of sufficient standing to give confidence to the Consular Authorities. Until the local Turkish Authorities and the consular representatives of the "Powers" can agree to work together loyally under the guidance of some authority in Public Health, acceptable to all, nothing can be done, for Jerusalem is a city of which the majority of the inhabitants are foreign subjects; these are of all nations, but especially Russian, Austrian, German and English or American. Some German societies, Christian and Jewish, have been recently looking into this question, and last September they sent Prof. Mühlens, of the School of Tropical Medicine at Hamburg, with three assistants to see what was the real state of things here especially with respect to malaria. Some account of what he has found I am giving below. As a result of his report Geheimrat Pannwitz, M.D., Secretary of the "International Association for the study of Tuberculosis," was commissioned to come out to Jerusalem and look into the question of Public Health here from the administrative side. He came in November 1912 and conferred with the Turkish local authorities and the various Consuls, and at a meeting of local medical men, representatives of the various national- and religious-community interests here, he laid before us the following proposal. He in the first place sought the co-operation of all the leading medical men here in the working of a Bacteriological and Public Health Laboratory which is now being established. This Laboratory will afford all the medical men here opportunities for examination of blood, sputum, urine, faeces and other bacteriological material; Widal's and Wassermann's tests will be done, whenever needed. The work will not only be done gratuitously but every facility will be given of allowing those medical men interested in such work to participate in any department in which they wish to specialize. A house has been engaged for the Laboratory—conveniently enough for me almost opposite my hospital—and Prof. Mühlens with two fully qualified medical men and some of the lay assistants, are now at work. Another bacteriologist from Germany is shortly coming. It is intended that as far as possible the staff should eventually be international and it is hoped, though the initiation has come from Germany, that those interested in Palestine in other lands will come forward and help to support the Institute. Probably, if the scheme is fully developed, the Laboratory will have three or four distinct departments each with a specialist in charge. Malaria and allied tropical diseases, tubercle, syphilis and rabies are some of the chief subjects for specialization. One of the most important objects at this

stage is the education of the public by lectures as to what are the outstanding sanitary needs of Jerusalem and Palestine generally. Meanwhile Dr Pannwitz is hoping, through negotiations carried on in conjunction with the world-wide Anti-Tuberculosis Association, to get the various foreign governments concerned interested, so that eventually a general recognition may be given to the Public Health Institute, a step leading it is hoped to co-operation of all concerned in the improvement of the lamentable state of the city.

Ever since Prof. Mühlens came to Jerusalem it has been my privilege to be closely associated with this scheme and with the details of the work. Those who are abroad, far from bacteriological laboratories, and who in the busy round of routine work have been unable to pursue, despite often the keenest interest, the bacteriological work which becomes routine work to many a medical man in the large cities of England, will fully appreciate what a boon some of us feel this new laboratory to be. We have already had opportunities of obtaining a positive diagnosis in many cases which has been most helpful to treatment, and it is unnecessary to say in a medical journal for fellow medical men how much this daily assistance along the more strictly scientific side of our work means to us. I am proposing to publish a few short papers, chiefly of a clinical nature, upon the prevailing diseases of Jerusalem and Palestine. The absence of a scientific basis for diagnosis has in the past always made me hesitate when tempted to write but now, thanks to Prof. Mühlens' kind co-operation and encouragement, our clinical observations are being placed upon a surer footing.

I. *The malarial fevers of Palestine.*

It is probably largely the result of the great prevalence of malaria in Palestine that the indigenous population are so wanting in moral energy, and that all efforts at the permanent settlement of Europeans in this land have, till very recent times, proved a failure. The climate of Palestine, in all the mountain regions at any rate, is salubrious. On the higher ground the mean temperature in February, the coolest month, is about 46° F. and the mean during the four hottest months—July to October—varies between 68° F. and 75° F. It is exceptional for the summer maximum temperatures in the shade to be above 100° F. Although the long dry season when not a drop of rain falls from the beginning of May to the middle of September is trying to some people, to others it is enjoyable and all are braced up by the cooler winter months.

Almost all the heavy rain falls during December, January and February—March sometimes replacing one of these as one of the wetter months. The rain is not however excessive; the average in Jerusalem is a little over 26 inches in the season and the spells of rain are interspersed by days, and sometimes weeks, of brilliant sunshine. The one drawback to all these healthful climatic conditions is malaria. It is always in our midst and it is widely distributed all over the land. In the low-lying maritime plain, inland from Jaffa, Caesarea and Haifa, many districts are very malarious; the low-lying Jordan Valley, below sea level for two-thirds of its extent and descending to nearly 1300 feet below the Mediterranean at the Dead Sea shores, is so malarious, on account of its sustained tropical climate, that much of it is no better suited for settlement by Europeans than the most unhealthy parts of tropical West Africa. But even in mountain regions where, as at Jerusalem, we are 2500 feet above sea level, malaria is exceedingly common. I suppose it may safely be said that taking all its effects—immediate and remote—there is no disease in the land which does so much harm. The evil results fall particularly upon the poorer classes and of course especially upon the children. Among the classes more comfortably off where care is taken, mosquito curtains being the rule and mosquito-proof houses increasingly common and where slight attacks of malaria are at once treated with quinine, severe malaria is exceptional. In my own household we all use mosquito curtains, although the windows and doors are supposed to be mosquito proof, but I also give all my children small doses of quinine throughout the summer and autumn as a further precaution. Before I did this I found their education constantly interrupted by slight attacks of malaria.

As things are Jerusalem and most of Palestine are quite unfit for any kind of "colonization"—as is being attempted by Jews from many lands—and a large percentage of the indigenous inhabitants suffer for many weeks every year, while many of them are chronically anaemic from these annual attacks.

This to us medical men is the more exasperating because we know that most, if not all, the malaria at a city like Jerusalem could be eradicated by measures which are comparatively simple and require for their enforcement merely that a certain amount of authority be conferred upon an intelligent staff of health officers. That a city of so much interest to so many well-to-do people, Christians, Moslems and Jews, should continue from year to year to be an ever increasing mass of malarial infection—a danger both to visitors and residents—is a

standing disgrace. An international Board of Health such as is now suggested with a medical officer (not in practice) giving his whole time to the question might, if supported by the Government and the various Consuls, do very much to improve things.

A few years ago it was a mystery how malaria could be prevalent in Jerusalem. Before the discovery of the malaria-bearing properties of the *Anopheles*, Jerusalem was certainly not the kind of site one would expect to be ague-haunted. Such sites were usually described as low-lying, marshy, etc., and the "ague miasma" was supposed to rise from the soaking lands at sunset and carry the disease which was at that period described as of "telluric" origin. But when the mosquito theory was first promulgated it seemed again a mystery how we could have *Anopheles* here. These mosquitoes were described by the earlier observers as passing their larval existence in slow moving, semi-stagnant streams where green algae abounded, and indeed in such places they (*Anopheles maculipennis*) are plentifully found in the lowlands of Palestine. It was only slowly that we found that the source of all the mischief lay in our numberless cisterns where the *Anopheles*, even more readily than the *Culex*, delights to dwell. Dr Cropper¹ showed that four species of Anophelidae were to be found in Palestine, viz. *Anopheles maculipennis*, *Pyrethrophorus palestinensis*, *Myzorrhynchus pseudopictus* and *Cellia pharoensis*. Prof. Mühlens now finds that the common *Anopheles* of our cisterns is an unspotted winged mosquito—common all over Europe—*Anopheles bifurcatus*.

The autumn months (Sept. Oct. and Nov.) are emphatically the "fever" months and the spring-season is by far the healthiest, but malaria, among the poorer classes, is never absent.

Among our out-patients the percentage of fever cases, which from January to June (inclusive) varies from 16 % to 21 %, runs up, during August, September, October and November to 40 % and over, falling again in December to 26 %.

In the same way among our in-patients out of a total of 1078 cases 289 or 27 % (excluding children) were fever cases (20 % in females and 36 % in males). Among the children in the childrens' ward the percentage was 50 %. During January to June the percentage (adults only) is 20 % and under 10 % in April, whereas during September, October and November the percentage runs 43 %, 50 % and 50 % respectively. If the children are included the percentage will be 18 %.

¹ Cropper, J. (1902), The Geographical distribution of *Anopheles* and Malarial Fever in Upper Palestine, *Journal of Hygiene*, II. p. 465.

in April and 55 % in October and 56 % in November. The three very malarious months are thus September, October and November, and with the commencement of winter there is a sudden drop.

I append a chart which shows graphically the percentage of *all* malaria cases (in- and out-patients, adults and children) in the different months and also the mean atmospheric temperature. The contrast of the two curves is very striking.

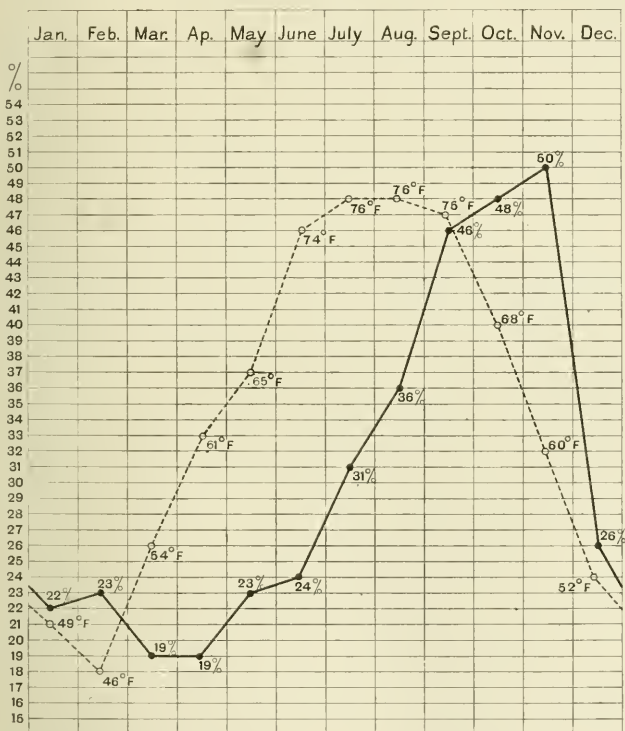
For many years these facts have been familiar to us but now, thanks to Prof. Mühlens, we have a great deal of new information which is of the utmost importance. Prof. Mühlens has examined schools and hospitals during the autumn months. In some of the Jewish day-schools he finds the astonishing percentage of 29 % to 30 % infected with malaria among the children actually attending the schools. If the absentees had been also counted the percentage would have been nearer 50 %. One such school at the time of the investigation had one-third of its scholars away—mostly for “fever.” In the boarding schools it is very much less. The larger number of these cases are “tropical” or “subtertian” fever, but there is a considerable quantity of quartan malaria also. Quartan fevers are very common and reach a high percentage of all cases in the winter months because of their persistence.

Prof. Mühlens will doubtless make a full report later on with regard to his general results based on the examination of several thousand cases, but I venture to give with his full permission my personal experience with my own cases. Altogether he has examined blood films which I have taken from over 700 of my out- and in-patients, and of these from September 26 to the end of November, I have through his kindness a full report on 666 cases. (It must be remembered that this by no means represents all the cases attending the out-patient department. Many have latent malaria and many were taking quinine regularly. Only scarcity of time limited my film-taking to likely cases.) Of these 317 were subtertian (tropical) malarial alone, 75 were quartan, 55 tertian, 9 were double infections of tertian and tropical, 4 quartan and tropical and 2 tertian and quartan. Of the total cases 69.3 % proved to have parasites and of these 71 % had tropical or subtertian malaria. This shows a terrible infection. Of the cases with gametocytes 116 were tropical (with crescents), 66 quartan and 52 tertian. Many of these cases had their blood literally crammed with parasites.

Seeing that we have in Jerusalem a very large percentage of human beings peculiarly liable to malaria—young European children and old

enfeebled European Jews just arrived with but little resistance to a tropical disease—a large percentage of gametocyte bearers and plenty of *Anopheles*, it is not surprising that we have abundance of malaria.

Percentage of cases diagnosed as "fever" cases among the in-patients and the out-patients at the English Mission Hospital, Jerusalem.



The black dots mark the percentage of "fever" cases in each month and the circles the mean atmospheric temperature for each month in Jerusalem. The "fever curve" is very characteristic and I have obtained the same taking out- and in-patients separately and with totals instead of percentages.

The infection of new cases with subtertian malarial parasites can hardly take place after the middle of November when the cooler weather sets in. The temperature is too low for the development of the sexual generation in the stomach of the mosquito, but the gametocytes die hard and with 36 % of all the subtertian cases with crescents in the blood, there is certainty that with the first return of the sustained warmth of summer the disease will be re-propagated.

With regard to the quartan and tertian cases there may still be re-infections during the winter. An investigation is being made during all the cooler months of this winter to see what forms of fever there occur and how many attacks are recrudescences of fever in cases of latent malaria. During December out of a total of 48 of my cases showing parasites 23 (or nearly 50 %) were quartan, 8 (14 %) were tertian, and only 20 (42 %) were subtertian: of these last 5 (25 %) had crescents only.

That there is an immense amount of latent malaria is shown by the large proportion of patients who, though exhibiting no outward manifestation of malaria, get a severe attack of ague immediately after an operation or after child-birth.

I have found much interest since this investigation was begun in comparing the clinical symptoms with the verdict of the microscope and certainly the latter upsets all the ordinary ideas of clinical experience. Scarcely one of the quartan or tertian cases could have been diagnosed from their charts—morning and evening temperatures only—even before any quinine was given: indeed the only quartan case which I had in the last two months with what might be called a “typical” chart was one which showed no parasites at all in the blood in the apyrexial stage. The subtertian cases had many of them no rise of temperature at all, or very little, although the blood was sometimes crammed with parasites: in the case of some infants the only clinical symptoms are anaemia, enlarged spleen and diarrhoea. High temperatures occurred with no parasites and normal temperatures with many parasites. Doubtless some of this latter class would be found to have rises of temperature if it were taken as is done, according to Prof. Mühlens, with such cases in Hamburg, every two hours day and night. It is true that in most of the subtertian malarial cases the spleen was enlarged, often markedly so, but on the other hand I have notes of a number of cases—during these two months alone—when from the spleen and the general anaemia a diagnosis of malaria seemed probable but no malarial parasites could be found, in some cases after two or three independent examinations.

Many of such cases have a perfectly normal temperature. It seems to me that many of these cases of pernicious anaemia, occurring not uncommonly in pregnant women, with very large spleens, but no malarial parasites, call for further investigation. There is a strong suspicion, on clinical grounds, that kala-azar is endemic in Palestine, but so far we have no pathological evidence.

Haemoglobinuria (blackwater) fever is now well known in Palestine. I believe one of my cases which I reported¹ was the first to be recorded in the land but it is now recognised everywhere. It cannot be called common in Jerusalem, but every autumn a few cases occur, and that too among patients who have not been outside the city for years. In the maritime plain, around Jaffa and in the malarious regions near Caesarea, it is far from uncommon. The worst region of all is the Jordan Valley near Lake Huleh and south of the Sea of Galilee, in both of which places many of the Jewish colonists have succumbed and others have been reduced to states of extreme anaemia.

A few words may not be out of place regarding Prof. Mühlen's methods, even at the risk of describing what must to some be very familiar. From all our patients we take blood from the lobe of the ear: I have myself found the best instrument to be a bayonet-pointed surgical needle, kept, fixed to the cork, in a small bottle of alcohol. From all the cases we make two slides—Slide *A* is an ordinary thinly spread film, Slide *B* is a thick film, two or more large drops being collected and allowed to dry towards each end of the slide. All the slides are numbered by means of a diamond glass cutter, and the numbers are recorded, in the first instance, against the names in my out-patient register. Slide *B* is stained *unfixed* with Giemsa's stain, one drop of the liquid stain to 1 c.c. of pure water; the solution is allowed to lie upon the slide for 20 minutes and is then washed off, after tilting off the excess, by gently dipping the slide two or three times in a beaker of pure water. The slide is then stood almost upright and allowed to dry: no cover glasses are used. The leucocytes and the malaria plasmodia take the stain, but the erythrocytes, if normal, are transparent; polychromatophilia is however so common that in a large percentage of the slides the erythrocytes show granular and faintly bluish. Slide *A*, if needed for more detailed examination of the blood-cells or parasites, is *fixed* in a mixture of alcohol and ether and then stained as above. As a result of the fixing the erythrocytes become sensitive to the blue stain.

To me the method of working with thick films has been a revelation: the examination of cases of suspected malaria, which by the old method of thin films often took hours, is now made with certainty and accuracy in a few minutes.

¹ Masterman, E. W. G. (1906), Haemoglobinuric Fever in Syria and some notes on the occurrence of the disease in Palestine, *Brit. Med. Journ.* 1. 314.

II. *Dengue.*

During the autumn months of 1912 a severe epidemic of dengue fever visited Syria. So far as I can gather it reached its height in Beirut and Haifa early in the past summer; it crept down the coast to Jaffa and later on to Gaza. From Haifa it passed inland to Nazareth and Tiberias and from Jaffa it spread into the mountains, devastating Jerusalem and its environs, spreading thence to Hebron, and, passing across the Jordan Valley where at least one large Bedoin tribe suffered severely, to *es Salt*. It would indeed appear to have visited all the large centres of population in turn. The disease reached Jerusalem somewhat late in the season; it was not very prevalent until the latter part of September, and it reached its full height in the city within-the-old-walls during the middle of October; during November it slowly diminished and now in the first week of December it has almost disappeared, though occasional new infections still occur.

The characteristic points of this disease have been briefly as follows:

A sudden onset—although this is not constant. In at least a considerable proportion of cases the patients describe the chill with which the fever commenced as being similar to an ague attack; it is often prolonged to three or four hours and some patients say they have had an actual shivering attack. Even when the cold stage is not so marked the patient becomes acutely prostrated in a very few hours and looks most alarmingly ill. From the onset headache and severe pain in the loins and in the hepatic and splenic areas are marked. Vertigo is common. The rheumatic pains in the limbs seem to be situated in the muscular and tendonous structures and not in the synovial membranes. Many cases have marked pain in the occipital region and the back of the neck. The eyes are congested and the eye-balls painful. Epigastric distress, ascribed to the heart, is very marked. There have been, particularly in this epidemic, marked gastric disturbances. Vomiting has been very common and often very severe indeed. The tongue, gums, fauces and pharynx are all congested. The tongue is usually found completely coated, from edge to edge, with an almost even, whitish, fur, which turns brown later. Constipation, often very obstinate, is very common. A good many cases have diarrhoea, which, often no doubt, is a result of a succession of purges taken one on the top of another for the relief of the constipation; some cases have had dysenteric attacks. The pyrexial stage seems to be anything from

three days in the mildest cases, to eight or ten in the severe. In the latter temperatures of 104° to 105° F. are common. I have not followed enough cases with the clinical thermometer to witness the regular relapse and recrudescence of pyrexia which is certainly characteristic of many cases. The final termination of pyrexia is frequently accompanied by profuse and prolonged perspirations. Epistaxis is so common at this stage as to be rather a symptom than a complication. In several cases it has, in my experience, been severe. I have also seen cases with haemorrhage from the throat, stomach, rectum or uterus. Abortions are said to be common but I have not met with one. After the fall in the temperature the pains, diminished in violence as a rule, often continue for many days or even weeks. The patient is much debilitated; perspirations may continue to be very troublesome. Two forms of rashes occur in the disease—(1) a severe congestion of the skin very noticeable in the face, which occurs at the onset of the disease in many severe cases, and (2) a much more frequent morbiliform or scarlatiniform eruption which appears at the end of the acute stage. Even this characteristic rash is not constant but will be seen in most cases if looked for. Its onset and sometimes its disappearance is frequently accompanied by an intolerable itching. Although during the period of the epidemic I must have attended many hundreds of cases I did not see one where death could be ascribed to this disease itself, but severe nervous prostration and neuralgia were very common, and some of my colleagues said they had seen old people succumb to the after effects. In one case I saw a severe attack was followed by a peripheral neuritis for which there was no other evident cause. The disease is not only often greatly prolonged, but relapses have been common, and from the experience of those who have lived long in Jaffa I gather that the occurrence of the disease gives little or no immunity. The epidemic simply dies out with the onset of cooler weather. One point worth noticing is that in the experience of most medical men here very young children, under, say, five, were seldom attacked, and in the doubtful cases when the mother had the disease the baby also had a temperature, the latter was found on microscopic investigation to have subtertian malaria and not dengue.

Dengue fever, or *Abu rikub*, has been more or less endemic upon the Syrian coast for some years, Manson¹ states since 1861. I understand that the last epidemic in Jerusalem was in 1889, but in Jaffa and along

¹ Manson, Sir P., Article "Dengue" in Allbutt's *System of Medicine*, Vol. II. Pt. II p. 345.

the coast dengue is a frequent visitor. In 1909 there was a severe epidemic and some cases coming from there at that time developed the disease in Jerusalem. Dr Keith of Jaffa says he has seen four epidemics, the usual season being September and October. The present epidemic began there about the end of August. Dr Torrance of Tiberias tells me that the fever visited that town in October and November and that probably quite 50% of the population were affected. In diagnosing our cases some of us had the co-operation and assistance of Prof. Mühlens, and through this we were able to eliminate the malarial cases. In the blood films from the cases which we clinically diagnosed as dengue marked leucopenia was usually present.

In making the diagnosis of dengue two other diseases, besides malaria, have to be excluded, viz. influenza and three-days fever. The differentiation may be difficult and in the beginning of an epidemic may be impossible in isolated cases. In influenza the onset is usually more insidious, the pains, on the whole, more severe, the respiratory organs are more frequently involved. In this dengue epidemic I did not meet with one case of pneumonia directly traceable to this disease. The heart and nervous complications are more marked and lasting in influenza; recurrence is less common, the relapses, in mild cases, less marked and the rash is absent. I confess that I called my earlier cases "influenza" (which is a common visitant in Jerusalem) because I was inclined to suppose that dengue, which I knew was raging at Jaffa, was a disease of the low land and that the only cases we were likely to get in the mountains (2500 feet above sea level) would be importations; then seeing the rash I suspected two concurrent diseases, influenza and dengue, but I am now sure that we have had an epidemic of pure dengue.

From three-days fever¹, dengue may be fairly clearly distinguished by the following points: (1) The former is rather a disease of summer, the latter of autumn. (2) The range of temperature is usually lower in three-days fever (100°-101° F.). (3) Three-days fever is characteristically of "three days" duration; relapses are uncommon. (4) Rashes are at least exceedingly rare, though they are described in three-days fever. (5) Vomiting, so common in this epidemic of dengue, is uncommon in three-days fever, while cough with a thick muco-purulent expectoration is frequent in the latter and is not naturally a symptom of the former. (6) Three-days fever confers an immunity after a time,

¹ Castellani and Chalmers (1910), "Three-days fever" in *Manual of Tropical Medicine*, p. 796.

second attacks are rare, third attacks are said to be unknown. It is a disease which attacks new comers to a district infested with sand-flies. Many tourists here get a slight attack after a visit to Jericho and some may also acquire it in Jerusalem where the *Phlebotomus pappatasi* is common. It never spreads through a community, attacking almost every adult, as this epidemic of dengue has done.

With regard to the ultimate cause of dengue fever we know little. It has been found by Ashburn and Craig¹ of the Philippine Islands that the organism must be smaller than even that of Malta fever, for infected blood can be filtered through a Berkefeld filter and still give rise to an attack of the disease if injected into a patient. It must be some organism comparable in minuteness to that of measles, small-pox, etc. There is a strong consensus of opinion that the bodies described originally by Dr Graham² of Beirut—"small hyaline, unstained dots or rods in the red corpuscles"—are not the cause of the disease. Dr Ardati, Dr Graham's pathological assistant, claimed to have stained them. "In specimens stained according to Giemsa-Romanowsky I was able to find in the erythrocytes, small, usually round, but sometimes elongated fine granulated, from purple to blue bodies of the size of $\frac{1}{5}$ to $\frac{1}{3}$ of a normal erythrocyte, occupying the margin but also at times the centre of the blood corpuscle³." Whatever these bodies may be they cannot be, if Ashburn and Craig's observations be correct, the real cause of dengue. One is strongly tempted to believe that Dr Graham's bodies are the same as those shown me some years ago by Dr Cropper, which he found in great numbers in many fever cases and which he then considered were the cause of a new disease, "Syrian fever," but which, I understand, he subsequently considered to be merely due to physical changes in the erythrocytes. Where stained bodies were found they may have been malarial parasites. Prof. Mühlens after carefully examining a great many of our blood films from a series of cases of dengue fever, in all stages, was unable to find anything answering to the description of Dr Ardati, *i.e.* any stainable bodies in the erythrocytes other than malarial organisms.

There is a general consensus of opinion that Dr Graham is right in his idea that the carrier of the disease is a mosquito, but I know of no evidence which proves that the *Culex fatigans* is necessarily the only carrier. If it is a minute organism of which the insect is the mere

¹ Ashburn and Craig (1907), *Philippine Journ. of Science*, II. 9.

² Graham, *Journ. of Tropical Med.* VI. 209.

³ Ardati (1910), *Medical Record*, Sept. 3.

carrier and there is no special development inside the mosquito—as with the malaria plasmodium in the stomach of the *Anopheles*—I do not see why *Anopheles*, sand-flies and even bugs may not carry the infection equally well. As culicine mosquitoes are by far the most conspicuous and generally diffused of such insects they may well be the chief and most frequent cause of infection with dengue in Syria. Persons living in mosquito-proof houses here appear to have escaped infection. Certainly all experience here points against infection by mere propinquity. If it is true, as our clinical experience seems to indicate, that very young children are much less liable to the disease than adults, then there must be another unknown factor in operation which needs investigation.

A MODIFICATION OF DIPHTHERIA ANTITOXIN.

By A. T. GLENNY, B.Sc.

(Preliminary Communication.)

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ONE of the fundamental features of Ehrlich's method of standardising diphtheria antitoxic serum is the recognition of the so-called L_0 and L_+ limits, the L_+ dose being the quantity of a toxin which when mixed with one unit of antitoxin and injected subcutaneously into a guinea-pig leaves sufficient excess of toxin free to cause the death of the animal about the fifth day after injection, the L_0 dose being the largest quantity which when similarly mixed with one unit of antitoxin fails to produce a local reaction. In the region between the two doses, the so-called "Differential-Region" of Ehrlich, one can obtain a series of mixtures which, while they kill the guinea-pig later than the fifth day or not at all, cause local reactions of varying severity increasing as the L_+ limit is reached.

It should be noted that Ehrlich draws attention to the fact that the oedema produced by these intermediate mixtures is far less severe than that produced by doses of unmixed toxin, even when these are small fractions of the M.L.D., and that it practically never terminates in extensive skin necrosis as does the reaction to toxin alone.

In the standardisation of an ordinary specimen of diphtheria antitoxic serum, when graduated doses are mixed with one test dose of toxin, the local and general effects obtained are of the type shown in Table I.

For a given toxin the volume of any normal diphtheria antitoxic serum, necessary for complete neutralisation of one test dose, bears a fairly constant ratio to the volume of the same serum which, when

mixed with one test dose, leaves one fatal dose free; in other words, to the volume containing one unit of antitoxin. This ratio corresponds to that between the L+ and Lo doses of the toxin in question. In the example given above the ratio would be as 1·2 is to 1·0.

TABLE I.

	Units of antitoxin	Toxin	Local reaction	Death
	0·95	1 test dose	Very large swelling	Death on 3rd day
L+mixture	1·00	„	Large swelling	„ 5th day
	1·05	„	„ „	„ 10th-20th day
	1·10	„	Moderate swelling	Survival
	1·15	„	Small swelling	„
Lo mixture	1·20	„	No swelling	„

The purpose of this note is to call attention to the behaviour of certain sera, the effects of which upon the action of toxin differ from the above type in that they possess in a relatively high degree the property of neutralising the power of diphtheria toxin to produce local reaction, but have comparatively little effect upon its lethal power. The serum exhibiting this anomaly in the most marked degree was obtained under the following conditions.

In the course of a study of the rate of deterioration in unit value of diphtheria antitoxic serum at various temperatures over a long period, the result of which I hope shortly to publish, I have had occasion to test serum which has been kept for seven years at 37° C. Table II shows the results of tests of a certain serum after different periods at this temperature. The serum has been kept in hermetically sealed phials and is sterile. The initial unit value was 440 units.

These tests were made against a toxin (J. 967) with average minimal lethal dose 0·004 c.c., Lo 0·33 c.c., and L+ 0·40 c.c., and injections of mixtures of fresh serum and this toxin usually gave rise to local reactions if less than 1·2 units of antitoxin were present in the mixture with one test dose of toxin.

The history of other batches of serum which have been kept for a considerable period at high temperatures is very similar and one more example is given in Table III.

It will be seen that such sera have no true Lo value and consequently the "differential region" no longer exists.

Progressive stages of modification are now being traced in a batch of horse serum kept at 37° C. and tested every few months. Successive tests have shown a diminution in size of the local reactions caused by

L+ mixtures, and it is hoped that one may be able to trace the gradual reduction of the "differential region" until the mixture with just enough serum to produce no local reaction is identical with the L+, and further modification until such a mixture becomes acutely lethal.

TABLE II.

Diphtheria antitoxic serum (Series E 148). Original value 440 units.

Tests made at the end of $4\frac{1}{2}$ years at 37° C.

Serum	Toxin	Local reaction	Death
1/200 c.c.	1 test dose	No record taken	2 days
1/100	"	"	3 "
1/75	"	No swelling	7 "
1/50	"	"	20 "

Tests made at the end of $6\frac{3}{4}$ years at 37° C.

1/100 c.c.	1 test dose	No record taken	2 "
1/75	"	"	2 "
1/50	"	No swelling	3 "
1/40	"	"	3 "
1/30	"	"	13 "

Tests made at the end of 7 years at 37° C.

1/60 c.c.	1 test dose	Very small swelling	2 "
1/50	"	No swelling	2 "
1/45	"	"	$2\frac{1}{2}$ "
1/40	"	"	3 "
1/35	"	"	7 "
1/35	"	"	9 "
1/30	"	"	16 "
1/30	"	"	17 "
1/25	"	"	16 "
1/25	"	"	23 "
1/20	"	"	26 "
1/10	"	"	Survival

TABLE III.

Diphtheria antitoxic serum (Series 469). Original value 470 units per c.c.

Tests made at the end of $4\frac{1}{2}$ years at 37° C.

Serum	Toxin	Local reaction	Death
1/75 c.c.	1 test dose	No swelling	3 days
1/50	"	"	8 "

Tests made at the end of $6\frac{3}{4}$ years at 37° C.

1/60 c.c.	1 test dose	No swelling	3 days
1/50	"	"	7 "
1/40	"	"	15 "

Diphtheria Antitoxin

The cases quoted so far are those of very old sera kept under special conditions until the unit value (judged by the L+ dose) has fallen very low. This modification of antitoxin, although most frequently observed in such old sera, is not confined to them alone.

TABLE IV.

Tests upon samples of blood from guinea-pig T 3. 7. 12 actively immunised to diphtheria toxin.

	Date of removal of sample of blood	Volume of blood	Volume of toxin	Local reaction	Death	
	21 Aug.	1/7 c.c.	0.01 c.c.	Very large swelling	Death in 4 days	
	25 Sept.	"	0.01	Large swelling	Survival	
	1 Oct.	"	0.01	Small swelling	"	
	4 "	"	0.02	Very large swelling	Death in 4 days	
	21 "	1/70 c.c.	0.01	" "	" 5½ days	
	23 "	"	0.02	No swelling	Survival	
	23 "	"	0.05	Large swelling	Death in 8 days	
	25 "	"	0.05	No swelling	Survival	
Modification of antitoxin	25 "	"	0.1	"	Death in 19 days	Probable period of maximum immunity
	28 "	"	0.1	"	Survival	
	28 "	"	0.15	"	Death in 16 days	
	28 "	"	0.2	Small swelling	" 3 "	
	30 "	"	0.2	No swelling	" 3 "	
	1 Nov.	"	0.1	"	Survival (paralysis)	
	1 "	"	0.15	"	Death in 13 days	
	5 "	"	0.15	"	" 3 "	
	6 "	"	0.1	"	" 20 "	
	11 "	"	0.12	Small swelling	" 4 "	
	21 "	"	0.08	Large swelling	" 4 "	
	2 "	"	0.05	"	" 5 "	

Among routine tests upon the unit value of fresh antitoxic serum from horses, instances have been noted in which the local reactions caused by an L+ mixture were smaller than those usually observed. More frequently this phenomenon has occurred when testing the blood of guinea-pigs actively immunised to diphtheria toxin. These tests were made during an investigation still in progress by Dr H. J. Südmersen and myself. Table IV records the most marked instance that has occurred. At frequent intervals blood was withdrawn from the animal, mixed with diphtheria toxin, and injected subcutaneously into other guinea-pigs. For a time these tests followed the normal course, producing local reactions; then for a certain period marked modification of the antitoxin occurred; later the test resumed its normal course. It

should be noted that the modification of the antitoxin occurred round the period of maximum immunity.

Mention may be made of modified antitoxin appearing in human blood, but the case is still under investigation.

CONCLUSIONS.

The properties of the above mentioned sera seem to warrant two conclusions:

1. The constituent of diphtheria toxin which is acutely lethal in its action is not identical with that which causes the local reaction at the seat of injection.

2. The power of a serum to neutralise the acutely lethal constituent of a toxin may vary independently of its power to neutralise the constituent causing local reaction.

The possibility that antitoxin as well as toxin may be complex has already been suggested (*e.g.* by Pick and Schwoner, *Zeitschr. f. exp. Path. u. Ther.* I. p. 98, 1905), but, so far as I can trace, no evidence has yet been recorded of the existence of two forms of diphtheria antitoxin possessing different affinities for the lethal constituent of toxin and for that which causes local reaction. I hope that the continued study of the action of modified sera upon toxins of different ages will give further information upon the constitution of diphtheria toxin.

NATURAL VARIATION OF *B. ACIDI LACTICI*
WITH RESPECT TO THE PRODUCTION OF
GAS FROM CARBOHYDRATES.

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Introduction.

VARIATIONS in bacteria may be said to fall into two classes (1) those which are temporary, and dependent on the environment, the bacteria tending to return to the original type when subculture is made on ordinary media; and (2) those which are fixed, and persist after repeated subcultures on ordinary indifferent media.

Although, for most purposes, this classification is useful, it is not always easy to decide to which class a given variety belongs. The altered culture may rapidly revert to the original type, because it consists of a mixture of the new variant and the original form, and ordinary media may exercise a selection which rapidly reduces the new variant to an imperceptible minority, or may lead to its complete extermination; while on the other hand growth on the medium on which the variant first appeared, favours the new form.

There may be a mixture of varieties of this kind in a culture which springs from a single variant bacillus, for though the majority of the bacilli produced belongs to the new variety, a small proportion of the original type often occurs by reversion in the course of successive generations.

That the proportion of variants in a culture depends on the medium on which the culture has been made and on the age of the culture was shown by Penfold (1911) in the case of cultures of the *Bacillus typhosus*

in dulcitate-peptone water in which the proportion of bacilli capable of fermenting dulcitate increased progressively.

Penfold also found that the variety of *B. coli* which had lost the power of producing gas from sugars after growth on chloracetic agar, did not remain constant till selection on the latter medium had been repeated. This tendency to revert in the earlier phases of the variant culture, indicated the presence of a mixture of the individuals which produced gas, and of those which did not, and perhaps also of intermediate variants.

The intermediate strains of the bacillus whose occurrence is about to be recorded in this paper, appear to have consisted of similar mixtures, though derived from colonies which in all probability arose from single bacilli.

Only that variety which remains constant on ordinary culture media can be considered as a true fixed variety, but even in this case the constituents of the medium may have a special selective action in some direction, and unless no atavist variant bacilli make their appearance in the course of growth the strain may revert to the original type.

The tendency of a variant to revert in culture, therefore, depends on the occurrence of individual bacilli of the original type and also on the favouring of these forms by the culture medium, but, frequently, atavistic bacilli may occur without an obvious change in the culture because the medium does not afford them any preferential treatment. On the other hand, if the particular medium is strongly selective for any particular type, the culture will in time take on the character of this type if individuals of this variety occur, even in small numbers.

Variations which remain constant in artificial culture have been described affecting many of the characters and biochemical properties of bacteria, but I propose here to mention only those relating to the fermentation of the carbohydrates.

Variations in fermentation.

Among the characters which are used for distinguishing allied forms of bacteria of the coli-typhoid group the production of acid, or acid and gas from sugars and alcohols, has proved to be of great service since these characters are as a rule uniform for known pathogenic bacilli both when the organism is first isolated and even after long growth and repeated subcultures on ordinary laboratory media.

Variations in fermentation reactions are not only of practical importance for diagnostic purposes, but are also of great theoretical interest,

since they affect some of the characters, which have usually been reckoned among the more permanent features of bacteria, and concern the physiological processes intimately connected with the life and growth of the bacteria.

Varieties showing changes in the fermentation characters during artificial culture have been described and fully tested of recent years.

Hiss (1904) found that *B. dysenteriae* Type Y could acquire the power to ferment maltose by growing on a medium containing that sugar.

Twort (1907) produced similar changes in *B. typhosus* as regards dulcitate and lactose.

Leutz (1909) and others have made similar observations.

Arkwright (1909) recorded natural and cultural changes in the fermentation properties of the meningococcus, which occurred independently of growth on sugars.

Penfold (1911, 1912) studied variations of this type in detail and has summarised the work on bacterial variation generally. He also confirmed and extended the work of Massini (1907) on *B. coli mutabile*.

Baerthlein (1912) correlated changes in the sugar reactions with variations in the type of colony on agar plates.

These examples, from amongst a large number of observations, illustrate for the most part the variations which have been observed in the direction of acquiring additional characters.

Other observations, which are no less striking, have been made on the loss or partial suppression of some characters.

Scheierbeck (1900) showed that streptococci occurring in milk vary very much in the amount of acid which they produce in a given time, and he was able by growing cultures in milk containing carbolic acid to obtain strains with widely different acid-forming powers, which remained constant after frequent subculture.

Penfold (1911, 1912) showed that it was possible to suppress to a very large extent the gas-forming property of *B. coli*, *B. enteritidis* (Gaertner) and *B. acidi lactici* by growing these organisms on agar containing chloracetic acid. The new variety in this case while forming acid from the same sugars and alcohols as the original strain had lost the property of forming gas from sugars, but retained it for the alcohols. He proved the identity of the two varieties by means of serological tests. The variants remained constant through numerous subcultures.

Revis (1911) by growing *B. coli* (Escherich) on 0.1% malachite green broth succeeded in suppressing the gas-forming function for alcohols and sugars, the variant still producing acid from the same sugars and alcohols as the original strain.

Natural variations.

Reference to the writings of workers on the subject of variation of bacteria, shows that while under special conditions definite and fixed varieties can be observed to occur, still there is little direct evidence to show that similar variation takes place under natural conditions, *e.g.* in the human body or in the outside world.

Colonies of the same organism but of dissimilar appearance occasionally appear on the original plates used for isolation, but when subcultures are made these differences usually disappear.

There is however some evidence (Baerthlein 1912) that fixed varieties which can be distinguished by the appearance of the colonies may be observed on the original plates inoculated with the stools of a patient.

The proof that these different types of colony consist of the same organism, is often imperfect, and in many of these cases proof of recent origin from a single strain is impossible and the same patient may have been infected from two different sources.

The acid-producing variety of *B. coli mutabile* which first appears on lactose agar subcultures has not as yet been conclusively demonstrated in the faeces. It is therefore only known to arise under artificial conditions.

Penfold (1912) however showed that the lactose-fermenting variants which are produced by *B. coli mutabile* closely resemble those lactose fermenters which commonly occur in the faeces.

It is more frequently in later subcultures that variations have been noticed, and since the best evidence of variation has been obtained by dealing with pure cultures from a single colony, most attention has been turned in this direction.

The natural varieties of pathogenic bacteria which may be isolated from different patients or in different epidemics, *e.g.* the morphological varieties of the *B. diphtheriae* described by Dale (1910) and the well-known subgroups of the mannite-fermenting type of the *B. dysenteriae*, belong to a different category, since it cannot be known how long their special characters have been acquired nor whether they first appeared in the patient from whom they were isolated.

In this connection must be mentioned the two following observations:

Bock (1906) found that three strains of *B. suispestifer* from different laboratories formed no gas from glucose, but as regards agglutination behaved like normal strains. He used malachite green agar ($\frac{1}{100}$ and $\frac{1}{500}$) for differentiation but does not state whether these strains had grown on this medium.

Bainbridge (1909) examined a bacillus which had been sent to him as a strain of *B. suispestifer* and found that it never formed gas from carbohydrates, although it formed acid from the same media as other strains, and in all other respects, including agglutination, resembled the standard *B. suispestifer*.

Nothing is known of the circumstances under which these varieties have arisen.

When bacteria have been met with, which present characters suggesting that they are natural variants of well-known types, direct evidence of the origin of the variant from the type form is usually absent or unconvincing. For instance, strains of bacteria belonging to the coli-typhoid family which resemble members of the *B. coli* group in producing acid from lactose, and in not liquefying gelatine, but which produce no gas from the sugars from which acid is formed, have been described by a few writers.

Mair (1906) recorded two strains of a coliform bacillus isolated from the urine of two patients which formed acid but no gas from glucose and lactose.

Wilson (1908) retested Mair's strains. He also examined 50 varieties of coliform organisms from the urine of cases of cystitis, pyelitis etc. Three of these latter strains formed acid but no gas from glucose. Including Mair's two bacilli he investigated five such strains. Of these five, two formed acid from the same sugars and alcohols as *B. acidilactici*, with the exception of adonite, and also like it formed indole in broth. One of the two latter strains formed acid and gas from mannite and sorbite, but only acid from glucose, lactose and other sugars, and therefore resembled Penfold's chloracetic variants.

I have been able to find a record of only one instance in which two varieties of a bacillus, which differ in little except in the production and non-production of gas, have been isolated from the same patient under circumstances which make it practically certain that the two varieties are in reality of identical origin and have become differentiated in the body of the patient.

The case referred to is that described by Sørensen (1912) who at different times isolated two strains of a coliform bacillus from the urine of a glycosuric patient. The two strains gave identical cultural reactions except that the one (Strain I) formed gas from all the sugars and alcohols from which acid was produced, and the other (Strain III *a*) formed acid only and no gas from these substances. (See Table I.)

TABLE I.

Sørensen's Bacillus	I	III <i>a</i>	III <i>b</i>	III <i>c</i>	IV
Glucose	A. & G.	A.	A.	A. & G.	A. & G.
Galactose			A.	A. & G.	A. & G.
Fructose			A.	A. & G.	A. & G.
Mannose			A.	A. & G.	A. & G.
Starch			A.	A. & G.	A. & G.
Maltose			A.	A. & G.	A. & G.
Lactose	A. & G.	A.	A. & G.	A. & G.	A. & G.
Cane sugar	A. & G.	A.	A.	A. & G.	A. & G.
Inulin			A.	A. & G.	A. & G.
Raffinose			A.	A. & G.	A. & G.
Mannite			A.	A. & G.	A. & G.
Milk	A.	A.	A.	A. & clot	A. & clot

There was evidence moreover that these two varieties exhibited the same differences in the patient's bladder. On the occasions on which the gas-forming variety (I) was isolated, the patient's bladder contained gas, and when the variety (III *a*) which did not form gas in culture was present, the gas in the bladder was not observed.

Sørensen showed the identity of the two strains by fermentation and cultural reactions but not by serum tests. Some differences were observed in the growth in broth. The gas-former grew with a slimy surface film on the medium and the bacilli grew in long threads, whereas the slimy character was absent in the other variety and the bacilli were shorter.

After growth for a month in artificial culture the variety of bacillus which at first formed no gas suddenly reverted to the gas-forming type (III *c*) as a sequel to growth on a 2% glucose medium.

At one period a culture (III *b*) with intermediate characters occurred in the course of cultivation on artificial media. This intermediate variety was distinguished by the formation of gas from lactose, but not from glucose or other sugars nor from alcohols.

At a later date the patient's bladder was again found to contain gas, and a gas-forming strain (IV) like Strain III *c* in every particular was isolated.

Strains I, III *c* and IV resembled each other in that they formed a slimy pellicle on broth; III *c* and IV differed from the other strains by clotting milk.

NATURAL VARIATION OF A BACILLUS OF THE *B. ACIDI LACTICI*
GROUP.

The bacillus which is the subject of the present communication was isolated from the urine of a man of 79 years who was suffering from an enlarged prostate and a variable degree of cystitis.

This bacillus, which belongs to one of the subgroups of *B. coli*, has been isolated from the urine every time that it has been examined, *i.e.* eight times in the 11 months from Feb. 1912 to 16 Jan. 1913. Though the bacillus presents variations in its characters, it is believed to be essentially the same throughout.

The urine has been passed into a sterile vessel and examined immediately. At first both staphylococci and streptococci were present as well as the coliform bacillus, but no streptococci were found at the last seven examinations and only a few staphylococcus colonies appeared on the cultures.

The deposit obtained by centrifuging the urine was examined in stained films and by plating out on agar. The bacillus was always found in large numbers by both methods.

Characters of the bacillus.

The bacillus isolated at the first examination of the urine in February 1912 had the same characters as the *B. acidi lactici* (Hueppe) as described by MacConkey (1909) except as regards motility. The typical *B. acidi lactici* is non-motile, but the bacillus now described is slightly motile in the sense that a few motile individuals can be found in six hours' and 24 hours' broth cultures. It therefore more accurately corresponds to No. 1 on MacConkey's list. It may perhaps for purposes of classification be called a motile variety of *B. acidi lactici*.

In morphology the bacillus varies in length but in young agar cultures it is frequently very short and almost coccoid.

The tests used on each occasion on which it has been isolated have been, growth on gelatin, litmus milk, peptone-water containing 1% of glucose, cane-sugar, lactose, dulcitol, mannitol, adonitol or inulin, and Ehrlich's test for indole in broth cultures.

On the original agar plates used for isolation, the colonies have usually been very small and translucent for 24 to 48 hours, but later and in subcultures large semi-opaque flat colonies have developed.

On the last seven occasions during the four months Sept. 1912 to Jan. 16, 1913, on which the bacillus has been isolated, several colonies have been examined from each specimen by the above mentioned tests. A few colonies have been grown on a more extended series of carbohydrates, glucosides and alcohols, as will be detailed later. (See Table III.)

The result of these examinations has been that two distinct varieties of the bacillus have been isolated, and in addition cultures showing intermediate characters have frequently been obtained by picking off single colonies from the original agar plates.

TABLE II.
Varieties of bacillus.

	I	II	III	IV
Glucose	A. G.	A.	A.	A. G. S.
Lactose	A. G.	A.	A. G.	A. G.
Cane sugar	-	-	-	-
Inulin	-	-	-	-
Mannite	A. G.	A.	A. G.	A. G.
Dulcitol	-	-	-	-
Adonite	A. G.	A.	A. G.	A. G.
Milk	A. C.	A. C.	A. C.	A. C.
Indole	+	+	+	+
Motility	+S.	+S.	+S.	+S.
Gelatin	-	-	-	-

A.=acid. A. G.=acid and gas. A. C.=acid and clot. +=indole positive. +S.=slight motility. -=no change in reaction of carbohydrates, or no liquefaction of gelatine.

I and II represent the two extreme varieties, III and IV the intermediate varieties.

Colonies on the original plates have, as a rule, been uniform in appearance and, when some difference in size or opacity has been noticed, these differences were not correlated with a different behaviour on test media.

No coliform bacillus giving other reactions has ever been found and the only other bacteria discovered have been staphylococci and previously to September 1912 also streptococci.

As is shown in Table II the only difference between the varieties has been in the production of gas, except perhaps some difference in

length of the bacilli and occasionally in the size of the colonies in subcultures.

Cultures of variety I sometimes show a longer form of bacillus than is found in similar cultures of variety II.

The two extreme varieties (I and II) have almost always bred quite true in artificial culture both when frequently subcultured and when left without subculture for upwards of three months on agar.

Evidence of an irregular change in an old agar culture has been noticed once and has latterly been obtained by growth in special media.

The intermediate varieties differed from the extremes only in details of the gas-producing function. They differed somewhat among themselves as regards the same character, some forms producing no gas from glucose but a full amount from lactose, while others produced a very little gas from glucose which was not increased after a week's incubation.

Some colonies produced only a very little gas from mannite.

TABLE III.

	I	Ia	II	A	B	C
Glucose	A. G.	A. G.	A.	A.	A.	A. G.
Laevulose	A. G.	A. G.	A.	A.	A.	A. G.
Galactose	A. G.	A. G.	A.	A.	A.	A.
Inulin	-	-	-	-	-	-
Dextrin	A. G. S.	A. G. ?	A. G. ?	A. G. ?	A. G. ?	A. G. ?
Maltose	A. G.	A. G.	A.	A.	A. G. S.	A. G.
Lactose	A. G.	A. G.	A.	A.	A. G. S.	A. G.
Cane sugar	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-
Arabinose	A. G.	A. G.	A.	A.	A. G. A.	A. G.
Salicin	A. G.	A. G.	A.	A.	A. G.	A. G.
Amygdalin	-	-	-	-	-	-
Isodulcitate	A. G. S.	A. G. S.	A.	A. G.	A.	A.
Mannite	A. G.	A. G.	A.	A.	A.	A. G.
Dulcitate	-	-	-	-	-	-
Sorbite	A. G.	A. G.	A.	A.	A. G. S.	A. G.
Adonite	A. G.	A. G.	A.	A.	A. G. S.	A. G.
Erythrite	A. ?	-	-	-	-	-
Inosit	-	-	-	-	-	-
Glycerine	A. G.	A. G.	A.	A.	A. G.	A. G.
Milk	A. C.	A. C.	A. C.	A. C.	A. C.	-
Indole	+	+	+	+	+	+
Motility	+ S.	+ S.	+ S.	+ S.	+ S.	+ S.
Gelatin	-	-	-	-	-	-

A = acid. A. G. = acid and gas. A. G. S. = acid and slight gas. A. C. = acid and clot.
Indole + = indole formed. Motility + S. = slight motility. Gelatine - = no liquefaction.

Moreover these intermediate forms showed a great tendency to change into variety I which produced a full amount of gas, or occasionally into variety II.

Three strains, namely I and Ia, which formed gas, isolated respectively in February and Sept. 1912, and II, a strain which did not produce gas, which was isolated in Sept. 1912 at the same time as Ia, were inoculated on 22nd December, 1912 into peptone water tubes containing 20 different carbohydrates, glucosides and alcohols. (See Table III.)

No differences between the three strains were found except as regards the production of gas. Strains I and Ia however always produced gas when acid was formed, whilst strain II produced only acid and never gas from any of the substances with the possible exception of dextrin, but in all the dextrin tubes small bubbles of gas appeared and the acid reaction was slight even in the case of Strains I and Ia.

All the strains formed acid and clot in milk, produced indole in broth, and grew on gelatine at room temperature without causing liquefaction.

At the same time three intermediate strains, A, B and C, which had been recently isolated (on Dec. 9, 1912), were inoculated into peptone water containing the same 20 test materials. These latter strains proved to be identical with the three strains I, II and Ia as regards their acid-forming properties, but varied amongst themselves as to gas production.

Strain A formed no gas, B very little gas and C much gas when grown on glucose peptone water immediately after isolation.

Serum tests.

Serum experiments were also made in order to test the identity of the three strains I, II and Ia. Three rabbits were inoculated with pure cultures of the different strains, and their sera used for agglutination tests and also for testing the absorption of agglutinins by the different strains. The results as seen in Tables IV and V showed no differences of any note between the three strains. No sign of agglutination appeared with normal rabbit's serum in dilutions of $\frac{1}{200}$ to $\frac{1}{12800}$, nor in 0.85% salt solution. The agglutination was carried out at the room temperature and was complete in 20 hours.

For comparison three laboratory strains of *B. acidi lactici* (Hueppe) were also tested as regards agglutination. With serum prepared from

TABLE IV.

Agglutination.

<i>Serum I</i>	1/400	1/800	1/1600	1/3200	1/6400	1/12800	(Control salt solution)
Bac. I	††	††	††	††	††	†	-
Bac. II	††	††	††	††	††	†	±
Bac. Ia	††	††	††	†	+	-	±
<i>Ser. Ia</i>							
Bac. I	††	††	††	††	††	†	-
Bac. II	††	††	††	††	††	†	-
Bac. Ia	††	††	††	††	†	±	-
<i>Ser. II</i>							
Bac. I	††	††	††	††	††	:	-
Bac. II	††	††	††	††	††	†	-
Bac. Ia	††	††	††	††	†	†	-
Bac. ac. l. 1	-	-	-	-	-	-	-
Bac. ac. l. 2	-	-	-	-	-	-	-
Bac. ac. l. 3	-	-	-	-	-	-	-

†† = complete. † = nearly complete. + = marked. ± = very slight.

- = no agglutination.

TABLE V.

Agglutination after absorption of agglutinins.

<i>Ser. I (abs. with Bac. I)</i>	1/800	1/1600	1/3200	1/6400	1/12800
Bac. I	±	-	-	-	-
Bac. II	+	+	-	-	-
Bac. Ia	±	±	-	-	-
<i>Ser. I (abs. with Bac. II)</i>					
Bac. I	+	±	-	-	-
Bac. II	+	+	±	-	-
Bac. Ia	±	±	-	-	-

TABLE VI.

<i>Ser. II (abs. with Bac. I)</i>	1/800	1/1600	1/3200	1/6400	1/12800
Bac. I	††	†	+	-	-
Bac. II	††	††	†	-	-
Bac. Ia	†	†	+	-	-
<i>Ser. II (abs. with Bac. II)</i>					
Bac. I	††	††	†	-	-
Bac. II	††	††	†	±	-
Bac. Ia	†	†	+	-	-

(Bac. I and Ia, gas-formers. Bac. II, non-gas-former.)

Bacillus I all three strains agglutinated fairly well in dilution of $\frac{1}{3200}$ and very slightly in $\frac{1}{3200}$. With serum prepared from *Bacillus II* no agglutination occurred in a dilution of $\frac{1}{800}$ nor in higher dilutions.

The agglutination of typical laboratory cultures of *B. acidi lactici* was therefore slight with Serum I and very slight or absent with Serum II. This evidence as far as it goes points to a closer relationship between the three strains I, II and Ia than between any of them and the laboratory strains.

The two sera I and II prepared with strains I and II respectively were used for absorption experiments and each serum was absorbed with each of the two bacilli separately. The results given in Tables V and VI show an approximately equal absorption by the different bacilli, of the agglutinins for all three strains.

The complete uniformity of the sugar tests as regards acid production and the strong confirmation of the identity of the three strains given by the agglutination tests leave little room for doubt as to the identity of origin of the varieties which form gas and those which do not.

It appears almost conclusively proved that they all originated from a single strain.

Transformation of one variety into another.

As a further test of the identity of the two varieties, attempts were made by culture to transform one variety into the other.

After many failures it was eventually found possible to transform the non-gas-forming variety into the variety which produced gas from sugars. Thus completing the proof of the essential unity of the two varieties.

Cultures of the two extreme varieties remained constant for over four months when subcultured at irregular intervals on agar or broth. In order to obtain information as to the media which favoured either variety, mixtures of the two forms were made by introducing drops of broth cultures of the two varieties into tubes of broth in different proportions, and subculturing daily after incubation into glucose and lactose peptone water. On subculture from mixed cultures after 18 days incubation gas was produced from lactose but little or none from glucose. When 1% glucose tubes which had been inoculated with mixtures in broth, were incubated and subcultured daily in glucose and lactose, after incubation for 48 hours, only acid and no gas were produced in the sugars. A similar result was obtained in subcultures after four days, but on the fifth day the subcultures failed to grow.

Glucose tubes containing pure cultures of the gas-former were also sterile after four days incubation. This and similar experiments showed that in glucose cultures the gas-former died out in four days to a week at 37° C., but the non-gas-former lived seven to ten days under the same conditions.

In lactose tubes inoculated with a pure culture of the gas-former, subcultures grew and showed production of acid and gas after at least eight days; and after at least five days in the case of mixed cultures, subcultures gave similar results.

It was found too that cultures of the intermediate varieties which when first isolated formed acid and no gas from glucose but acid and gas from lactose, changed so as to produce acid and gas from both sugars after growth in broth for two or three days, but after growth in glucose peptone water for a similar period sometimes no gas was formed from either sugar.

In accordance with these results attempts were made to procure a variation in the gas-former by plating out after three or four days' growth on glucose peptone water, and examining a number of colonies in expectation that if a non-gas-forming variant occurred it would be encouraged in the acid glucose culture in preference to the gas-former.

No such variant could be demonstrated by these means. Attempts were also made, so far without success, to select out a gas-former from a culture of the variety II by prolonged incubation in weak (0.05%) glucose broth or in ordinary broth, and also by repeated subcultures in ordinary broth continued for over a month.

Penfold (1911, 1912) found that his chloracetate variant of *B. coli* which produced no gas from glucose readily produced gas from sodium formate, showing that in all probability the peculiarity of this variant consisted in a defect in its power of making formic acid from the sugar.

He found, moreover, that by growing *B. typhosus* and his chloracetate variant together in glucose peptone water gas was formed which he attributed to the breaking down of the formic acid made by the *B. typhosus*.

Harden and Penfold (1912) subsequently confirmed this view by showing that the amount of formic acid produced by the chloracetate variant was much less than that produced by the original strain of *B. coli*.

In order to find out whether my gas-former (II) had similar characteristics as regards gas formation from formates, cultures were made with a loop of a broth culture of variety II (1) together with

B. typhosus in glucose peptone water, (2) in peptone water and in broth containing sodium formate with the following results:—

The gas-forming variety (I) produced abundant gas from peptone water containing sodium formate in 24 hours.

Variety II which did not form gas from sugars or alcohols was shown to be a slow gas-former from sodium formate. (See Table VII.)

Similar results have been obtained in broth containing larger percentages of formate.

TABLE VII.

One loop of broth culture of II (non-gas-former) added to each tube and incubated at 37° C.

Tube		Time	Result
1	1 % glucose peptone water + five drops of a <i>B. typhosus</i> culture	7 days	Acid, no gas
2	0.5 % sodium formate in peptone water	5 "	No gas
3	1.0 % " " " " "	5 "	Some gas
4	2.0 % " " " " "	5 "	No gas
5	1.0 % " " " broth	2 "	Some gas

TABLE VIII.

Cultures of variety II (non-gas-former) in media containing sodium formate subcultured to lactose.

Date of culture	% formate	Date of subculture to lactose	Result in lactose
24/1/13	2 % in peptone water	29/1/13	Acid on 30/1/13
		31/1/13	" " 1/2/13
"	1 % " "	29/1/13	" " 30/1/13
		31/1/13	" " 1/2/13
"	0.5 % " "	29/1/13	" " 30/1/13
		31/1/13	" " 1/2/13
"	1 % in broth	29/1/13	Acid and gas on 30/1/13 (small quantity)
		31/1/13	Acid and gas on 1/2/13 (large quantity)

It seemed possible that this late yield of gas was due to a "training" or selection of the bacilli which acted on the formate under suitable conditions of growth, especially in the absence of acid which appears to inhibit or destroy the gas-formers as shown above.

The cultures of II in formate (see Table VIII) were therefore subcultured to lactose peptone water with the following results:—

The 1% formate broth was also plated out on agar on the 31st Jan. and after incubation showed colonies of different sizes. Five large colonies when inoculated into lactose broth gave acid and no gas as before, but of nine small colonies inoculated into lactose peptone water, eight gave acid and gas after incubation for 24 hours. The gas-forming property still remained after subculture on ordinary nutrient broth.

These experiments appear to show that the defect in variety II associated with its inability to form gas is not a want of power to make formic acid but to an inability to split the formic acid formed under ordinary conditions of culture in glucose peptone water, and that the power to produce gas from formates may be acquired in a neutral solution of sodium formate in broth.

The new variant (II *a*) of variety II was examined by inoculation of the formate broth culture into a series of sugars and alcohols, and the identity of its reactions with those given by variety I was shown.

Further work is being done on these varieties.

Discussion of results.

There is no direct evidence in the case of the bacillus described above as to which variety should be considered the original parent and which the more recent variant. However, the fact that the gas-former corresponds in almost all its characters with the *B. acidilactici* which is very commonly met with in the faeces, makes it more probable that this is the parent form.

Variation in bacteria which is shown by the loss of some property has been attributed to a general lowering of the functional activity of the bacteria concerned, in which one function has been suppressed before the remainder, because it has been more recently acquired or is less essential to life.

Thus Scheierbeck's variant streptococci were produced by the inhibiting agent—carbolic acid—and the impaired acid-forming function of the bacteria was associated with slower growth.

Such an explanation, if cogent, would perhaps lessen the importance of lost characters from the theoretical standpoint of evolution, though for the practical purpose of diagnosis the loss and the acquisition of a characteristic are equally important.

The loss or impairment of a function may however be associated with a gain in another direction as in the case of Scheierbeck's streptococcus cultures which produced less acid during the first period

of growth, but continued to grow for a longer time in the cultures and eventually formed a higher percentage of acid than the more rapid acid-formers.

Penfold's variant on chloracetic-agar which had lost its power to form gas from sugars, grew in larger colonies on the selective medium than the original type, indicating an increased vigour rather than a diminished one.

One of the varieties of the bacillus which is the subject of this paper, had no power of forming gas from sugars or alcohols, and in some instances appeared to grow in smaller colonies on agar than certain races of the gas-forming variety. When however cultures of the two varieties were mixed in broth and the mixture plated out on agar the gas-former grew in smaller colonies than the variety whose power to form gas was in abeyance. This was shown by inoculating a number of the small and of the large colonies into glucose peptone water.

Moreover, the variety which did not form gas survived for a considerably longer period in glucose peptone water than the gas-former.

The suggestion, therefore, that a general weakness is the rule in the case of strains of bacteria which show loss of function in one direction, is by no means supported by the evidence in all cases, and the apparent loss may be fully compensated in other directions.

Some special features of the varieties described above remain to be mentioned.

(1) The power of producing gas from alcohols is absent in the case of those strains which have not this function in regard to sugars. In this respect the anaerogenic strain resembles those produced by Revis by cultivating *B. coli* on malachite-green, and also differs from Penfold's variant in that it does not readily produce gas from formates.

(2) The intermediate strains which I obtained from colonies on the original plates mostly showed the remarkable characteristic that they produced a full amount of gas from lactose in 24 hours, but none or very little from glucose in the same time. This behaviour is difficult to explain since *a priori* it seems probable that the lactose is first split into glucose and galactose before these sugars are further acted on with the production of acid and gas.

The suggestion that the gas was formed from the galactose split off from the lactose appeared to be negatived by the fact that when grown in galactose and in glucose, these strains yielded very small amounts of gas from both these sugars, but from lactose at the same time a large yield was obtained.

This observation however does not stand alone. Penfold (1911, 1912) found that during the selection of his chloracetate varieties, the power to form gas from glucose disappeared before the same function as regards lactose.

Revis also had the same experience. Sørensen too noted a somewhat similar phenomenon, as is shown in Table I.

(3) The relative proportions of the different varieties obtained from the samples of urine varied somewhat on different occasions. (See Table IX.)

Thus of 70 colonies picked off the plates made on the 8th December, 21 formed full gas, and 24 formed acid only, whereas 25 showed intermediate characters.

TABLE IX.

Number of colonies examined on each occasion.

Sample of urine...	R. 1 Date...12/9/12	R. 2 20/9/12	R. 3 10/10/12	R. 4 31/10/12	R. 5 8/12/12	R. 6 31/12/12	R. 7 17/1/13	Total
Full gas I	4	3	4	3	21	5	4	44
No gas II	2	1	4	3	24	15	14	63
Intermediate III. (No gas from glucose)	3	0	1	0	0	1	3	8
Intermediate IV. (Some gas from glucose)	3	2	0	0	25	3	9	42
	12	6	9	6	70	24	30	157

Of a total of 157 colonies picked off the original plates on the last seven occasions, 44 produced a large amount of gas from glucose, lactose and mannite; 63 formed no gas from any of these three substances and 50 were intermediate in character. Of these latter eight produced no gas from glucose, while the lactose tubes showed a large amount of gas in the Durham's tubes. The remainder formed very little gas from glucose but a large amount from lactose. In each case the gas production was judged by the gas collected in the small Durham's tubes.

It seems unlikely that so large a proportion as 50 isolated colonies out of 157 (31·8%) were formed from two bacilli of different varieties. These intermediate forms must therefore be regarded as having arisen from single bacilli of intermediate character or from bacilli which at once gave rise to individuals of varying type.

(4) It has not been found possible to maintain the intermediate strains constant. When plated on agar from broth, or glucose or lactose

peptone water cultures, the colonies when inoculated into sugar media usually conform to one or the other extreme type of variety, but occasionally a culture showing intermediate characters has been obtained after plating out the culture three times in succession.

CONCLUSIONS.

1. A bacillus belonging to the *B. acidi lactici* group has been repeatedly isolated during 11 months from the urine of one patient, and no other Gram-negative bacillus has been found in the same urine during this period.

2. The bacillus has occurred in two varieties which differed as regards gas-formation only. Variety I formed gas from sugars and alcohols and variety II formed acid and no gas from the same sugars and alcohols.

3. The two varieties gave identical serum reactions both as regards agglutination and absorption of agglutinins with specific sera prepared from rabbits immunised with the respective varieties.

4. Intermediate varieties as regards gas production also occurred, but were not constant when subcultured.

5. Varieties I and II remained constant in their characters after four months' subculture on broth and agar.

6. Variety II which at first did not produce gas from sugars was induced to do so by first growing in a solution of sodium formate in broth.

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ON THE NATURE OF THE CELLULAR ELEMENTS
PRESENT IN MILK. PART IV. THE HISTO-
LOGICAL APPEARANCES OF THE UDDER.

(FOR THE BRITISH DAIRY FARMERS' ASSOCIATION.)

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(With Plate III.)

IN former reports by us, considerable attention was devoted to a study of the number and nature of the cellular elements in milk. We found that cells, sometimes in enormous numbers, are present in all milk (cow, goat, ass, man), that considerable variations in number occur without apparent cause, and that the presence of the slighter forms of non-suppurative mastitis does not seem to influence definitely the number of these cells.

From a study of a large number of stained films of the cellular elements present in milk, the cells were divided into six groups: (1) *Large uni-nucleated* cells, probably epithelial cells derived from the secreting layer of the gland tissue; (2) *multi-nucleated cells*, regarded as being the "germinal cells" of Winkler; (3) *small uni-nucleated cells*; (4) *eosinophile cells*; (5) *vacuolated cells*, degenerate, fat-bearing or colostrum-like cells; (6) *cells of indeterminate nature*. No cells resembling polymorphonuclear leucocytes were detected, though the "multi-nucleated" cells might well be mistaken for them.

The present report deals with the histological appearances of the udder, sections of which have been studied in the hope that some light might be thrown on the nature and source of the cellular elements in milk.

Winkler (*loc. cit.*) is particularly emphatic that leucocytes are practically never found in milk (under normal conditions) and are never found in the lumina of the alveoli of the glandular tissue of the udder, and quotes Michaelis as expressing a similar opinion. Winkler regards the majority of the cells in milk as being epithelial cells detached from the glandular layer of the alveoli. He distinguishes a layer of "germinal cells" under the epithelial layer of the gland. These were first described by Kolessnikov in 1877, and a similar layer in the submaxillary gland was described by Heidenhain. These "germinal cells" are regarded by Winkler and others as embryonic epithelial cells; they grow upwards, enlarge, and develop into and replace the cells of the epithelial layer, as these are detached or become senile. They are delicate, rounded or ovoid cells with a well defined nucleus, which is sometimes double owing to division taking place, with a fair amount of cytoplasm.

The udders which have been examined are those of: (1) a normal goat; (2) a cow (Cow No. 6) which developed a slight non-suppurative mastitis; (3) another cow (Cow 37), which also developed a slight non-suppurative mastitis; (4) three other cows, which exhibited more or less tuberculous infection, but not of the udder.

The tissues were obtained perfectly fresh, and pieces were fixed in (a) Müller's fluid, (b) a saturated solution of mercuric chloride with acetic acid, (c) formalin, 10 per cent. After fixation, the tissues were well washed in water and hardened in alcohol, and sections were prepared after embedding in paraffin and stained with Ehrlich's haematoxylin and eosin. On the whole, the tissues fixed in Müller's solution gave the best results.

The following are the results of the examination:

Udder of goat.

The alveoli are on the whole small and contracted; the glandular epithelium is regular and cubical to columnar in shape. Free cells in the lumina of the alveoli are conspicuously absent. The aspect of the gland is that of the quiescent stage.

Udder of cow No. 6.

Cow No. 6 was one of the six cows of Dairy Farm C, the milk of which was critically examined as to the number and nature of the cellular elements present, and reported on in our second report. This cow while under observation developed a slight thickening of the right hind quarter, with lessened yield of milk, about August 8th 1909. By August 25 she had apparently recovered. She was slaughtered on February 14th 1910.

Sections from the four quarters were separately examined. Microscopically, there is little to distinguish one quarter from another—the size of the gland alveoli, the epithelial layer and the general appearance of the epithelium and of the inter-glandular tissue differ but little in appearance in the four quarters. In the right hind quarter, which was slightly abnormal clinically, there is no increase in the inter-glandular tissue, and no sign of infiltration with leucocytes or round cells.

Left Quarters. Alveoli moderately distended. Practically no free cells in the lumen of the alveoli. Multi-nucleated cells in the germinal layer practically absent.

Right Fore-quarter. Similar to the left quarter, except that a few free cells (uni-nucleated) are present here and there in the lumen of the alveoli.

Right Hind quarter (the affected quarter). On the whole the alveoli are more dilated than in the other quarters; they are probably at their maximum distension. Many of the alveoli contain a homogeneous substance or a net work of fibrillated material, presumably the coagulated remains of the milk. A few large and small uni-nucleated cells are present free in the lumen of some of the alveoli. A few of the uni-nucleated cells described as of the “normoblastic” type are present in the inter-alveolar tissue in close association with the alveoli, suggesting plasma cells.

Sinus. In sections taken from another portion of the gland adjacent to the milk sinus very interesting details are shown. The alveoli are dilated and the glandular epithelium is swollen and the cells to a large extent are vacuolated. The picture as a whole suggests that this portion of the gland is at the height of its functional activity. Numerous alveoli contain from a few to many free cells in their lumina. These free cells are of several of the types described by us in the films prepared from milk, and confirm the views expressed as to the nature of some of

these cells. There are the large uni-nucleated cells, obviously detached glandular epithelial cells. There are cells in all stages from these to the large vacuolated ones, which are thus manifestly degenerate and sodden glandular epithelial cells. There are also some of the small uni-nucleated cells, and many of the multi-nucleated cells with two to four nuclei. A fair number of small uni-nucleated cells can be seen in the tissue under the glandular epithelial layer. The whole picture is strongly confirmative of the views of Michaelis and Winkler on the presence of the "germinal" layer of cells, and that the multi-nucleated and many of the small uni-nucleated cells present in milk are derived from this germinal layer as previously surmised by us.

Udder of cow 37.

Cow No. 37 was one of the several recently calved cows of Dairy Farm D, the milk of which was critically examined as to the number and nature of the cellular elements present, and reported on in our second report (*loc. cit.*). This cow was examined and reported on by Mr Villar on four occasions. On December 3rd 1909 Mr Villar found that she had a "fleshy" udder, and that the right hind quarter was slightly larger and more firm than the corresponding left quarter, though the milk was apparently normal in quality and quantity. She "ran her milk" from the left hind quarter. On December 24th 1909, the right hind quarter was quite normal, but the left hind quarter was now obviously swollen, though the milk was apparently normal in quality and quantity, and her temperature was 102.6 degrees. The condition was regarded as a non-specific interstitial mastitis. On January 19th 1910, Mr Villar found that the right hind quarter was normal, but in the left hind quarter the mastitis was slightly more marked; the quarter was somewhat harder but not larger and the first milk drawn from it was flaky and yellower than normal. The cow's temperature was still 102 degrees, and she coughed, and was obviously not healthy. On February 19th 1910, she was reported much better. She was slaughtered on March 11th 1910.

Fore Quarters. Alveoli distended. Epithelium very regular. Many free cells in some of the alveoli; they are mostly large and small uni-nuclears. A small number of multi-nuclears (2-4 nuclei) also present, but no cell resembling a polymorphonuclear leucocyte. In a section from HgCl₂-hardened material a number of these multi-nucleated cells were seen in the alveoli.

Left Hind Quarter (probably the most abnormal). The alveoli throughout are small and contracted. There may be some cellular infiltration in the inter-alveolar tissue, but it is difficult to be certain of this on account of the general contracted condition of the gland, *i.e.* this infiltration may be apparent only and due to a closer packing of the tissue between the alveoli. A few of the alveoli contain free cells of the small uni-nucleated and multi-nucleated types.

Right Hind Quarter. A condition similar to that of the preceding specimen is present, but is more marked. The glandular alveoli are to a large extent obscured, and there is an undoubted infiltration of the inter-alveolar tissue with round cells. In the alveoli which persist, a good many free multi-nucleated cells (2-3 nuclei) are present.

The three last udders examined were obtained from the slaughter-house through the kindness of Mr McPhail. They were all from cows suffering from more or less tuberculous infection, without, however, any clinical sign of disease of the udder.

Cow with tuberculous infection of the supra-mammary lymphatic gland.

The gland is largely fatty; no tubercles present. The glandular alveoli are relatively scanty and contracted. Here and there a small uni-nucleated cell is present free in the lumen of an alveolus. The gland is obviously atrophic and losing its functional activity.

Cow with generalised tuberculosis.

The glandular alveoli are moderately distended and the glandular epithelium is swollen and vacuolated, suggesting that lactation is either at an early stage or has recently terminated. Many free cells are present in the lumen of the alveoli; these are of the large uni-nuclear and multi-nuclear (2-4 nuclei) type. Large vacuolated cells are also present. The basement membrane is well seen, and many small uni-nuclear cells with deeply staining nuclei (*i.e.* the "germinal" cells of Winkler) are present in close approximation with the epithelial cells.

Cow with tuberculous infection of the supra-mammary gland.

The glandular alveoli are contracted and free cells in their lumina are absent. The gland is largely fatty and atrophic, and losing its functional activity.

CONCLUSIONS.

This examination of these several udders has shown the extreme paucity of polymorphonuclear leucocytes in the inter-alveolar tissue, and so far as can be seen their complete absence in the lumina of the alveoli. In the sub-epithelial layer, cells corresponding to the "germinal" cells of Winkler have been detected and lend support to his conclusions respecting the origin of the epithelium from these cells.

Within the lumina of the alveoli, cells of the (1) large uni-nuclear, (2) small uni-nuclear, (3) multi-nuclear and (4) vacuolated types have been found in some of the specimens, and a study of their appearances confirms our previous views on the nature of these cells, viz. that the large uni-nuclears and vacuolated cells are epithelial cells, and that the small uni-nuclears (generally) and the multi-nuclears are cells of the "germinal" layer. None of the eosinophile type has been detected, and their origin therefore remains doubtful.

The results of this examination confirm the opinion we have already expressed that the cellular elements found in milk, either normally or in ordinary catarrhal or interstitial non-suppurative mastitis are *tissue* cells, and that "pus cells," in the ordinary acceptance of the term, do not appear in milk under these conditions.

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DESCRIPTION OF PLATE III.

- Fig. 1. Section of glandular alveolus, showing a large uni-nuclear and small uni-nuclear cells free in the lumen.
- Figs. 2, 3. Sections of glandular alveoli, showing multi-nuclear cells free in the lumina.

PUBLICATIONS RECEIVED.

BOOKS.

ARMSTRONG, H. G. and FORTESCUE-BRICKDALE, J. M. (1912). *A Manual of Infectious Diseases occurring in Schools*. Bristol: John Wright & Sons, Ltd. 150 pp., 7 pls. 19 × 12 cm. Price 3/- net. Cloth.

This manual is primarily meant to be for the guidance of schoolmasters and mistresses. The diseases dealt with are clearly described. The authors have tried to avoid technicalities as far as possible, but even with the help of the glossary we should fancy that the lay reader would find it difficult to understand completely all the terms used. M.

CHAPIN, C. V. (1912). *The Sources and Modes of Infection*. (Second Edition, revised and enlarged.) New York: John Wiley & Sons. 481 pp. 21 × 13 cm. Price 12/6 net. Cloth.

This book is very well arranged and clearly printed. The first chapter discusses the very important subject of the life of germs outside the body, and the bearing of this subject on the spread of infection. In Chapter II a clear and interesting account is given of "carriers" and missed cases in all the more important diseases. The next chapter discusses the limitations and value of isolation. In the subsequent chapters infection by contact, fomites, air, food and insects is discussed. The book is well worthy of careful study by all interested in the spread of infectious diseases, whether medical practitioners, sanitary officers, bacteriologists, or members of Public Health Committees. The book is up-to-date and can be most thoroughly recommended. G.-S.

CLEMESHA, W. W. (1912). *The Bacteriology of Surface Waters in the Tropics*. Calcutta: Thacker, Spink & Co., 6, Mangoe Lane. 161 pp. 22 × 14 cm. Price 7/8 rupees. Cloth.

This book is the outcome of about 5 years' work carried out by the author in Madras and Bengal and it incorporates his report (1909) on the subject which appeared from the King Institute. Since the publication contains much original work it should prove of special interest to those who have to deal with water supplies in the tropics. N.

CURWEN, M. and HERBERT E. (1912). *Simple Health Rules and Health Exercises for busy Women and Girls*. London: Simpkin, Marshall, Hamilton, Kent & Co., Ltd. 47 pp., 31 figs. 19 × 12 cm. Price 1/- net. Boards.

This little manual contains some excellent health rules, while the physical exercises are carefully explained and rendered more easy to understand by reason of the numerous photographs which accompany the list. If only people who have to follow sedentary occupations would carry out rules and exercises of this nature, their health would be benefited considerably. M.

DELL, J. A. (1912). *The Gateways of Knowledge*. An Introduction to the study of the Senses. Cambridge: University Press. 171 pp., 51 figs. 20 × 14 cm. Cloth.

This book is an introduction to the study of experimental psychology, intended for class work in schools. It is written in an interesting manner, and provides a useful and at the same time entertaining manual for instruction in the physiology and psychology of the special senses. M.

GHOSH, B. N. and DAS, J. L. (1912). *A Treatise on Hygiene and Public Health. With Special Reference to the Tropics*. Calcutta, India: Hilton & Co., 109, College Street. 378 pp., 28 figs. 19 × 12 cm. Price 5/- net. Cloth.

This treatise deals mainly with hygiene in the tropics, and more particularly with the descriptions of food, the disposal of sewage and septic tanks. The chapter on infectious diseases is carefully written, and the latest information with regard to preventive measures is briefly detailed. The work does not claim to be original. G.-S.

GIBSON, I. G. (1912). *Medical Benefit in Germany and Denmark*. London: P. S. King & Son, Orchard House, Westminster. 296 pp. 22 × 14 cm. Price 6/- net. Cloth.

The author has produced a very valuable book on the medical aspect of State Sickness Insurance, in which he contrasts and compares the German and Danish methods, the former being a compulsory system and the latter a voluntary one. All those who are interested in the subject of medical benefit under the Insurance Act should read the book, particularly those medical men who have taken service under the Act. It is not necessary to agree with all the conclusions which the author draws from his comparative studies, though these, on the whole, are fairly warranted by the facts adduced, except in the case against a Public Service, in which the conclusions arrived at are obviously the outcome of preconceived opinion, and not founded on facts, since, as far as we are aware, no such system has at present been fairly tried. M.

HALDANE, J. S. (1912). *Methods of Air Analysis*. London: C. Griffin & Co., Ltd., Exeter Street, Strand, W.C. 130 pp., with 24 illustrations, including 1 plate. 24 × 13 cm. Price 5/- net. Cloth.

This little book contains a short and concise description of the methods which the author has found, from his personal experience, to be most serviceable for everyday needs in connexion with analysis of air and simple gas mixtures. Most of the methods are original; they have been described and previously published by the author.

We have nothing but praise for this little volume. The methods described are simple, clearly set out, and whenever necessary the apparatus is illustrated; the various problems are exemplified by simple calculations. The general arrangement of the matter is excellent, the style is easy and very free from technical phrases, and the print is large. This volume must form an important addition to the libraries of all chemists and physiologists who are interested in gas analysis. D.

HOLLINGWORTH, H. L. (IV. 1912). *The Influence of Caffeine on Mental and Motor Efficiency*. (Archives of Psychology, No. 22. Edited by R. S. Woodworth.) Columbia Contributions to Philosophy and Psychology, vol. xx. No. 4. New York: The Science Press. 166 pp., with 31 figs. 24 × 16 cm. Cloth.

This book forms the record of an investigation undertaken at the instigation of, and financed by the Coca-Cola Company of Atlanta, America. The experiments give every impression of being carefully and accurately carried out and leave no reason to suppose that they are in any way biased. We would however hardly agree with the statement in the preface that there is an absence of adequately reliable data on the action of caffeine on mental and motor processes, especially as in the course of the author's experiments he entirely confirms the results of Rivers that "caffeine increases the capacity for both muscular and mental work...without there being any evidence, with moderate doses of reaction leading to diminished capacity for work."

The experiments are clearly set out and form a convincing argument as to the action of caffeine. A useful summary is appended to the end of each chapter. D.

HOOKE, A. H. (1913). *Chloride of Lime in Sanitation*. New York: John Wiley & Sons. 231 pp. 23 × 14 cm. Cloth.

The sanitary aspects of disinfection with chloride of lime are carefully set forth in this volume, accompanied by numerous abstracts and references. It ought to be in the hands of all interested in sanitary matters. G.-S.

HUTT, C. W. (1912). *Hygiene for Health Visitors, School Nurses and Social Workers*. London: P. S. King & Son, Orchard House, Westminster. 415 pp., 71 illustrations. 22 × 14 cm. Price 7/6 net. Cloth.

This book, which is mainly written for persons interested in public health, but without special medical training, gives a clear and simply written account on many subjects, such as food, clothing, personal hygiene, care of infants and children, of which they should possess an intimate knowledge. This book ought to serve a most useful purpose, and deserves a wide circulation. G.-S.

JORDAN, E. O. (1912). *A text-book of General Bacteriology*. (Third Edition, thoroughly revised.) Philadelphia and London: W. B. Saunders Company. 623 pp., 164 figs. 23 × 14 cm. Cloth.

This excellent book has now reached the fourth edition, and contains within its 600 pages the most essential facts of bacteriology. It is well and carefully written, excellently printed, and for the most part clearly illustrated with reproductions and beautiful photographs. It is also up-to-date, and can be most thoroughly recommended as a text-book on the subject. G.-S.

LETULLE, M. and NATAN-LARRIER, L. (1912). *Précis d'Anatomie Pathologique*. Vol. I. Paris: Masson et Cie, Editeurs, Boulevard Saint-Germain, 120, 940 pp., 248 text-figures. 20 × 13 cm. Price (Cloth) 16 francs.

As stated in the preface this book is a "Précis," a condensed account of the essentials of human pathological anatomy both macro- and microscopical. The complete work will consist of about 2000 pages, all of the illustrations being original and most of the descriptions being based upon the author's own observations. We can but recommend this excellent treatise to our readers. N.

MCCARRISON, R. (1913). *The Etiology of Endemic Goitre*. London: John Bale, Sons & Danielsson, Ltd., 83-89, Great Titchfield Street, Oxford Street, W. 216 pp., 57 figs., 1 map. 22 × 14 cm. Price 10/6 net. Cloth.

This book presents in amplified form the five Milroy Lectures delivered by the author at the Royal College of Physicians of London in January, 1913.

Major McCarrison has brought together what is known to-day regarding the causation of endemic goitre, and, coupled with an excellent bibliography at the end of the volume, the book should prove of material assistance to students of the subject. The work is excellently illustrated by photographs of cases and of the microscopic lesions observed and by a map showing the distribution of goitre. The volume contains much work that is original. x.

- McKAIL, D. (1912). *Public Health, Chemistry and Bacteriology*. (A Handbook for D.P.H. Students.) Bristol: John Wright & Sons, Ltd. 409 pp. 19 x 12 cm. Price 6/6 net. Cloth.

This book is divided into two parts dealing respectively with Public Health, Chemistry and Bacteriology. In the chemical part are chapters dealing with the analyses of water, air, soils, foods, beverages and disinfectants. Each chapter is clearly and concisely written and the examples excellently chosen. In the bacteriological part are chapters dealing with general principles, results of bacterial activity, immunity and anaphylaxis, cocci, non-sporing bacilli, sporing bacilli, spirilla, spirochaetes, yeasts and moulds and special bacteriological examinations. The descriptions of the characters of the specific organisms are too short to be of much value in themselves. The book is not illustrated. The book is intended to assist in, and supplement, actual laboratory teaching, and not in any way to supersede it. G.-S.

- MASTERS, P. G. (1912). *Home Exercises and Health*. Five minutes care to the Nerves. London: John Long, Ltd., Norris Street, Haymarket. 144 pp., with 34 illustrations and 1 Chart. 19 x 12 cm. Price 2/6 net. Cloth.

The author claims for his system that it is calculated to improve the general health rather than to develop muscular powers. There can be no doubt that if everyone would devote a few minutes each day to systematically carrying out the exercises recommended, the physique of the nation would be immensely improved, but unfortunately people will not take the trouble to do this. M.

- MILES, E. (1912). *Fitness for Play and Work*. London: Thomas Murby & Co., 6, Bouverie Street, E.C. 110 pp., with Exercises. 17 x 10 cm. Price 1/6 net. Cloth.

A little book, written in a popular vein, which contains many good maxims and sound advice suitable for boys. x.

- PAKES, W. C. C. (1912). *The Science of Hygiene*. A Text-book of Laboratory Practice for Public Health Students. New Edition, revised by A. T. Nankivell, M.D., D.P.H. Methuen & Co., Ltd., 36, Essex Street, London, W.C. 164 pp., 80 figs. 20 x 13 cm. Price 5/- net. Cloth.

This book is intended for the Medical Officer of Health and the student for the Diploma in Public Health, and contains all the practical laboratory work it is necessary to know at the present time. The work is detailed under four heads—Bacteriology, Microscopy, Chemistry and Physics (including vital statistics).

The arrangement of the book is excellent and the examples given and the illustrations of the methods of interpreting results of chemical analyses should be of great value to students. While some of the sections are treated fully, others are poor, for example that relating to human parasites (p. 136). The illustrations of fleas and ticks are of little value, and in the case of the latter the text

is inaccurate and misleading. Bacteriological methods are omitted and no attempt is made to give the relationship of chemical to bacteriological methods in water analyses. G.-s.

RIVIÈRE, D. DE LA (1912). *Méningites à Pseudoméninocoques et Méningites à Paraméninocoques*. Paris: Imprimerie de la Cour d'Appel. Rue Cassette, 1. 114 pp.

The author's *thesis* is dedicated to some forty persons and is largely a compilation. It will be of use to those interested in the subject who desire a summary of our present knowledge or who wish to look up the literature, 160 references being given in the bibliography. N.

ROBERTSON, W. and McKENDRICK, A. (1912). *Public Health Law*. An Epitome of Law applicable to England and Wales and Scotland. Edinburgh: E. & S. Livingstone, Teviot Place. 397 pp. 18×12 cm. Price 5/- net. Cloth.

This book contains a most useful digest of the laws relating to public health matters. The important sections of the various Acts are separately and clearly dealt with, and an excellent index is added at the end. It ought to be a most useful work for ready reference. G.-s.

ROSS, H. C. (iv. 1912). *Further researches into Induced Cell-reproduction and Cancer*. London: John Murray, Albemarle Street, W. 125 pp., with illustrations. 22×14 cm. Price 3/6 net. Cloth.

This volume deals with researches on cell-proliferation and cell-development. It is well illustrated and clearly printed, and deserves careful consideration by those specially interested in these problems. G.-s.

STICKER, G. (1912). *Abhandlungen aus der Seuchengeschichte und Seuchenlehre*. II. Band: Die Cholera. Giessen: Verlag von Alfred Töpelmann (vormals J. Ricker). 594 pp., 4 text-figs. Price 30 marks.

The first volume of this monumental work, relating to plague, appeared in two parts in 1908 and 1910 (see notice in *Journ. Hygiene* x. 309), this, the second volume, treats of cholera: cholera nostras, cholera infantum and asiatic cholera. Following upon an introduction the subject matter falls into three sections dealing with each of these diseases, 90 pages are devoted to the first two and 421 pages to asiatic cholera. In each section the author discusses the history of the disease from the earliest records until to-day, its epidemiology, cause, clinical features, diagnosis, prognosis, prophylaxis and therapy. A bibliography of 63 pages and an index conclude the volume which should be consulted by all who have to deal with cholera. The book represents an enormous amount of labour and is the most exhaustive treatise which has hitherto appeared on the subject. N.

BROCHURES.

GLOGNER, M. (1912). *Die Nahrungsmitteltheorien über die Ursache der Beriberi in kritischer Beleuchtung*. 56 pp. Leipzig: J. A. Barth.

The author undertakes to show that beriberi is not a specific disease and bases his statements on a considerable experience acquired in the Dutch East Indies and elsewhere where the disease prevails. N.

LOVETT, R. W. and SHEPPARD, P. A. E. (1912). *Infantile Paralysis in Massachusetts* *Journ. of Hyg.* xiii

during 1910, together with Reports of special investigations made in 1911 bearing upon the etiology of the disease and the method of its transmission. (Reprinted from *Monthly Bulletins of the Massachusetts State Board of Health* for 1911.) Boston: Wright & Potter Printing Co., 18, Post Office Square. 154 pp. with Maps.

LUCAS, R. C. (1912). *The Bradshaw Lecture on some points in Heredity*. (Delivered before the Royal College of Surgeons of England, 6 Dec. 1911.) London: Adlard & Son, Bartholomew Press, Bartholomew Close, E.C. 50 pp., 8 figs.

PAYNE, E. H. (1912). *Public Baths and Bathing Places*. Suggestions on the general arrangements, structure, and equipment of Public Swimming Baths and Bathing Places. Published by the Southern Counties Amateur Swimming Association, Hon. Sec. H. E. Fern, High Barnet, Herts. Price 6d. 24 pp., 25 figs.

A short account of public baths and bathing places, their purpose and how they should be constructed, the text being accompanied by instructive plans and illustrations. Two appendices relate to the laws governing bathing establishments. N.

PEABODY, F. W., DRAPER, G. and ROCHEZ, A. R. (1912). A clinical study of Acute Poliomyelitis. New York: The Rockefeller Institute for Medical Research. *Monographs of the Rockefeller Inst. for Med. Res.*, No. 4. 187 pp., 13 pls.

SCHAEFER, R. (1912). *Bilden Volkshelstätten eine Gefahr für ihre Umgebung?* München: Rudolph Müller & Steinicke. 24 pp. Price 1.40 Marks.

The title of this brochure "Do public sanatoria constitute a danger to the locality in which they are situated?" constitutes the question which the author discusses and answers in the negative, basing his conclusions upon statistics relating to sanatoria for tuberculosis in Krailling, Neuried, Martinsried and Planegg. N.

TSUZUKI, J. (1912). *Antiberiberintherapie der Beriberikrankheit*. Leipzig: Verlag von J. Ambrosius Barth. 71 pp. Price 2.20 Marks.

The author, who belongs to the Beriberi Institute, Tokio, has had many years experience of the disease in Tokio where about 2000 deaths a year are attributed to it. He states that beriberi stands in intimate relation to rice diet, that the disease experimentally induced in animals is essentially similar to that in man and can be cured or prevented by similar means. He has found a remedy in the inner cortex of the rice grain which can be extracted by alcohol from rice and which he calls "antiberiberin." The latter can be administered *per os* or by injection and acts as a preventive or cure. He gives experimental evidence in support of his statements. N.

WALLACE, J. S. (1912). *The Prevention of Dental Caries*. Second Edition. London: The Dental Record Office, Abston House, Newman Street, W. 70 pp., and frontispiece. 14 x 22 cm. Cloth. Price 1/6 net.

The fact that a second edition of this little book has been called for within the space of six months is a proof of value attached to it by the Dental Profession. The author lays down sound principles as to diet in infancy and childhood. If these were universally applied in practice it is unquestionable that dental caries might be largely diminished with a corresponding improvement in the general health of the community. W. M.

REPORTS.

Annual Report (1912) of the Department of Public Health (Ministry of Interior), Cairo, for 1911. Paper No. 2—1912. Cairo: Government Press. 152 pp., with maps and diagrams.

This report deals with (i) Medical Administration: general provisions for medical aid and provisions for special departments.—(ii) Public Health: General Considerations; Infectious Diseases; Sanitary Defence; General Sanitary Measures; Municipalities and Local Commissions; Governorates and Provincial Councils.—(iii) Scientific Establishments.—(iv) Veterinary Department.—(v) Engineering Department.—(vi) Legislation. N.

BAHR, P. H. (1912). *Dysentery in Fiji during the year 1910*. (Report to the London School of Tropical Medicine.) London: Witherby & Co., 326, High Holborn, W.C. 77 pp., with coloured and monochrome plates, and many charts.

BASHFORD, E. F. (1912). *Fifth Scientific Report on the Investigations of the Imperial Cancer Research Fund*. London: Taylor & Francis, Red Lion Court, Fleet Street, E.C. 94 pp., 4 pls. Boards.

FLEXNER, A. (1912). *Medical Education in Europe*. Bulletin Number 6. (The Carnegie Foundation for the Advancement of Teaching.) Boston: D. B. Updike, The Merrymount Press. 357 pp.

Mr Abraham Flexner has sought to deal with Medical Education in Europe in a manner similar to that which characterized his previous report on Medical Education in the United States and Canada (Bulletin No. 4), with this difference that the present report deals only with a limited number of representative institutions in Germany, France and England, with the view of giving a picture of contemporary medical education in these countries. The report will be read with interest by all who concern themselves with the subject. N.

GRAHAM, W. M. (1912). *Report on Blackwater Fever in Southern Nigeria 1899–1911*. London: Waterlow & Sons, Ltd., London Wall. 72 pp., 4 plates, 4 Charts and 1 Map.

Following upon a discussion of the views held by various authors regarding the etiology of blackwater fever Dr Graham deals with the disease as observed by him in Southern Nigeria. Excellent photomicrographs illustrate the pathological lesions. N.

HAY, M. (1910). *Report by the Medical Officer of Health for the year 1910*. With Appendix on Still-Births in Aberdeen. (City of Aberdeen.) 113 pp.

HOLMES, J. D. E. (1912). *Report of the research work of the Imperial Bacteriological Laboratory, Muktesar, during 1910 and 1911*. No. 3. Calcutta: Thacker, Spink & Co. 276 pp.

HOUSTON, A. C. (H. 1912). *Eighth Report on Research Work*. (Metropolitan Water Board.) 18 pp., 1 Diagram. Metropolitan Water Board Laboratories: 20, Nottingham Place, London, W.

JATTA, M., LORIGA, G. and MAGGIORA, R. (1912). *La Tuberculosis nell' uomo e nei Bovini in Sardegna*. (Studio epidemiologico e sperimentale.) Roma: Tipografia delle Mantellate. 159 pp., 1 Map.

LISTON, W. G. (1912). *Report of the Bombay Bacteriological Laboratory for the year 1911*. Bombay: Government Central Press. 43 pp. Price 7 Annas, or 8d.

MOSS-BLUNDELL, C. B. (1912). *Annual Report of the County Medical Officer upon the Health and Sanitary condition of the County of Huntingdon for the year 1911*, compiled from the Reports of the District Medical Officers of Health. Huntingdon: D. Cooper & Co., Printers, High Street. 43+xxxvi. pp., with tables.

NASH, J. T. C. (1912). *Annual Report of the County Medical Officer of Health and School Medical Officer for the year 1911*. (Norfolk County Council.) Shirehall, Norwich: 137 pp.

PANNWITZ, Prof. Dr (1912). *Tenth International Tuberculosis Conference, Rome, 10th-14th April, 1912*. Berlin-Charlottenburg: Internationale Vereinigung die Tuberkulose im Selbstverlage. 501 pp. 23×21 cm. Cloth.

PIRAS, L. (1912). *Osservazioni Batteriologiche fatte durante il Colera di Genova del 1911*. (Published from the Ufficio d' Igiene del Comune di Genova, Laboratorio Batteriologico dell' Ospedale d' Isolamento.) Novi Ligure, Genova: Tipografia Cooperativa. 51 pp.

A report on the bacteriological examination of 1523 suspects and of 394 cases of cholera which occurred in 1911 in Genoa. There occurred two cases in June, 69 in July, 267 in August, 49 in September, five in October, and two in November. N.

PORTER, C. (1912). *Report of the Medical Officer of Health on the Public Health and Sanitary Circumstances of Johannesburg during the Two Years, 1st July, 1909-30th June, 1911*. With Appendices by (1) the Medical Attendant on the health of natives (P. S. Stock).—(2) Municipal Census (G. D. Maynard).—(3) Mortality amongst natives employed in Mines and Works (G. D. Maynard). Johannesburg: Adlington & Co., Printers, S. Africa. 85 pp.

PORTER, C. (x1. 1912). *Report of the Medical Officer of Health on the Public Health and Sanitary Circumstances of Johannesburg during the year, 1st July, 1911-30th June, 1912*. To which is appended a Report by the Medical Attendant (P. G. Stock, M.B., D.P.H.), on the Health of the Natives employed by the Council. Johannesburg: Adlington & Co., Printers, S. Africa. 57 pp.

Publications (1912) of the Civil Medical Service in Netherlands India. vols. 1 a 111 pp. with figs. and 1 b 151 pp. 1 pl. Batavia: Javasche Boekhandel & Drukkerij. Boards.

Vol. 1 a deals with the bacteriological diagnosis of plague at Malang (J. de Haan) and gives an extract of the Government Report on the plague epidemic at Malang, Java, during Nov. 1910 to August 1911 (W. H. Th. de Vogel); the epidemic was preceded by a plague epizootic among house rats. The volume is excellently illustrated with numerous maps and photographs and is published both in Dutch and English. Vol. 1 b relates to epidemiology of plague in Java: rats and their habits, their parasites, etc. (J. J. van Loghem); the plague in Karanglooe in May-July 1911 (A. A. F. M. Deutmann); extracts from the reports (O. L. E. de Raadt). This volume is likewise fully illustrated. The volumes contain many valuable and original observations. N.

Rapport à M. le Préfet (1912). *Sur les Recherches effectuées au Bureau du Casier sanitaire pendant l'année 1911 relatives à la répartition de la tuberculose et du*

cancer dans les maisons de Paris. Paris: Imprimerie et Librairie Centrales des Chemins de fer, Imprimerie Chaix. Rue Bergère 20. 132 pp.

The report deals with the distribution of tuberculosis and cancer in Paris. The statistics relating to tuberculosis, collected during 18 years, concern 169,705 cases and the houses in which they occurred. The Paris authorities have continued to suppress dark and unhealthy dwellings and especially since 1909 have they succeeded in condemning "des maisons meurtrières" on a more extensive scale with the result that in the three succeeding years the mortality from tuberculosis has been reduced by 2252 cases. The moral to be drawn from the report is to let in air and sunlight and destroy buildings which cannot be rendered healthy by attempts at reconstruction. The report is signed by M. Paul Juillerat (Chef du bureau administratif des services d'hygiène). N.

Report of the Surgeon General U.S. Army to the Secretary of War. (Annual Reports, War Department, 1912.) Washington, D.C.: Government Printing Office. 261 pp.

The report gives a full account of health and disease in the U.S. Army both at home and abroad. N.

Second Report (1912) of the Government Bureau of Microbiology, dealing with work performed during the years 1910 and 1911. Sydney: W. Applegate Gullick, Government Printer. 244 pp.

Sixth Annual Report of the Henry Phipps Institute for the Study, Treatment and Prevention of Tuberculosis (University of Pennsylvania), February 1, 1908–February 1, 1910. (Issued 1912.) Henry Phipps Institute: 238 Pine Street, Philadelphia.

Statistical Report (1912) of the Ambulatory Patients of the Quinton Polyclinic for Treatment by Isotonised Sea Water from July 1st to December 31st, 1911. London: The Quinton Polyclinic, 57, Poland Street, W.

Statistique Démographique des Grandes Villes du Monde pendant les Années 1880–1909. Seconde partie. Autres Parties du Monde et Annexe Générale (1912). Communications statistiques publiées par le Bureau municipal de Statistique d'Amsterdam. No. 40. Amsterdam: Johannes Müller. 115 pp. Price for both parts: 3 fl.

Statistische Mitteilungen (1912) veröffentlicht vom Statistischen Amt der Stadt Amsterdam. No. 38. Reproduktion der wichtigsten graphischen Darstellungen von dem Statistischen Amt Amsterdams vorgeführt auf der Internationalen Hygiene-Ausstellung Dresden, 1911, nebst erläuterndem Zahlenmaterial. Amsterdam: Johannes Müller. 41 pp.

Tenth Annual Report (1912) of the Imperial Cancer Research Fund (London). London: Taylor & Francis, Red Lion Court, Fleet Street. 12 pp.

Third Report (1912) of Deptford School Clinic or Health Centre for School Year, August 29th, 1911, to July 30th, 1912. London: P. S. King & Son, Orchard House, Westminster. 31 pp. Price 3d.

Twenty-first Report (1912) of the Board of Health on Leprosy in New South Wales, for the year 1911. Sydney: W. Applegate Gullick, Government Printer. 26 pp.

Verwaltungsbericht (x. 1912) des Magistrats zu Berlin für das Etatsjahr, 1911. No. 18. Bericht der Deputation für die städtischen Krankenanstalten und die öffentliche Gesundheitspflege. Berlin: W. und S. Loewenthal. 21 pp.

NEW JOURNALS.

Bibliographische Monatsschrift. Internationale Zeitschrift für die gesamte Literatur der Medizin. (Zentral-Organ der Medizin.) Editor: H. Albert-Hellmers, Hamburg. May, 1912. 108 pp. Vol. i. No. 1. Verlag der Internationalen bibliographischen Monatsschrift: Rettig und Kollmorgen, Hamburg 36. Annual Subscription (12 numbers): 36 Marks.

This new bibliographical review is intended to fill a want which the editor does not consider to be satisfied by existing publications including the well-known *Index Medicus*. The new bibliography does not give the full titles of the papers but it gives condensed titles in black type, these titles being ordered alphabetically according to their key words. An author's index accompanies each part. Books are marked with a star. Whilst appreciating the courage of the undertaking and the fact that the new bibliography may be of considerable use we hold the opinion that it is not an improvement upon the *Index Medicus*. We cannot refrain from expressing our regret that a new competitor to the *Index Medicus* should enter the field since such publications are not remunerative enterprises and can but react injuriously upon the limited circulation which it is possible for either of them to attain. x.

Zeitschrift für Gärungsphysiologie, Bd. i. Heft 1. (iii. 1912.) Edited by Professor Dr A. Kossowicz-Wien. Berlin: Verlag von Gebrüder Borntraeger. W. Schöneberger Ufer 12a.

Papers dealing with the physiology of fermentation and mycology have hitherto been scattered over a large number of journals; it is the object of the new Journal to collect them, so as to make them more readily available for workers on the subject. The journal will publish original papers, collective reviews, epitomes and lists of papers which have appeared in other places. It thus serves both as a journal and a Centralblatt, and will no doubt prove useful. The first number contains several interesting papers, and a useful review of recent advances in agricultural bacteriology. A.

PERIODICALS AND CONGRESS TRANSACTIONS.

Atti della Società per gli Studi della Malaria, vol. xii. 580 pp., with figs. 1912. Rome: Società per gli Studi della Malaria.

Contains 31 papers by various authors describing the antimalaria campaign etc. conducted in different parts of the world. x.

British Guiana Medical Annual for 1910. (1912.) Edited by K. S. Wise, M.B., B.Sc., B.Sc. (17th year of issue.) Demerara: "The Argosy" Company, Ltd. 126 + xevi pp. with plates and photographs. Price 5/-.

Contains papers on various diseases, etc., occurring in British Guiana: Enteric Fever, by E. D. Rowland; Midwifery, by A. J. Caigen; Village sanitation, by A. T. Ozzard; Ankylostomiasis, by G. E. Carto; Nastic treatment of Leprosy, by K. S. Wise; Anthrax in Man, by E. P. Minett; *Culicis mimeticus*, by K. S. Wise; Leprosy Conference, by J. E. Godfrey; Clinical notes, by Q. B. de Freitas; Public Health Statistics, etc. x.

Congrès (1912). *Mondial des Associations Internationales, Bruxelles*, 9-11 Mai, 1911. Volume i. Documents Préliminaires, Rapports. Bruxelles: Office Central des Institutions Internationales. 3 bis, rue de la Régence. pp. 1-830.

- Congrès (1912). *Mondial des Associations Internationales, Bruxelles, 9-11 Mai, 1910*. Volume II. Procès-Verbaux des séances. Bruxelles: Office Central des Institutions Internationales. 3 bis, rue de la Régence. pp. 831-1246.
- Hong-Kong Medical Congress. *Transactions of the Second Biennial Congress of the Far Eastern Association of Tropical Medicine* held at Hong-Kong, 1912. Hong-Kong: Naronha & Co. 399 pp., with pls. and figs. 25 x 16 cm. Price 10/6. Cloth.
- Contains papers dealing with Beri-beri, *Entamoeba*, Cholera, Tuberculosis, *Distomum*, Plague, Surgery, Syphilis, Sunlight, Care of Children, Tetanus, Relapsing Fever, Malaria, Anophelines and their parasites (*Ceratopogon*), Blackwater, etc., in the tropics. N.
- Proceedings (1912) of the Canal Zone Medical Association* (Isthmian Canal Commission) for the half-year April to September, 1911. Vol. IV, Part I. Mount Hope, Canal Zone: Isthmian Canal Commission Press, Quarter-master's Department. 238 pp.

REPRINTS.

- ANDERSON, J. F. and FROST, W. H. (1912). Transmission of Poliomyelitis by means of the Stable Fly (*Stomoxys calcitrans*). Washington, D.C.: Government Printing Office. Reprinted from *U.S. Public Health Reports*, No. 99, 5 pp.
- BÖESEKEN, J. und WATERMAN, H. I. (VI. 1912). Ueber die Wirkung der Borsäure und einiger andren Verbindungen auf die Entwicklung von *Penicillium glaucum* und *Aspergillus niger*. *Folia Microbiologica*. 1 Jahrg, Heft 3, 17 pp.
- CHRISTOPHERS, S. R. (1912). *Malaria in the Andamans*. Calcutta: Superintendent Government Printing, India. *Scientific Memos. by Officers Med. & Sanit. Depts. Gov't of India*, N.S. No. 56. 48 pp., one photograph. Price 14 Annas, or 1/4.
- DELÉPINE, S. (1912). Bovine Tuberculosis. London: H. & W. Brown, 20, Fulham Road, S.W. Repr. from *Proc. Nat. Veter. Assoc.*, 13th Ann. Meeting, Manchester, VII. 1912. 10 pp.
- DELÉPINE, S. (1912). The share taken by Human and Bovine Tuberculous Products in the infection of Young Children. London: Adlard & Son, Bartholomew Press, Bartholomew Close, E.C. Reprinted from *Trans. 4th Ann. Conference Nat. Assoc. Prev. Consumption and other forms of Tuberculosis*, Manchester, 5th, 6th and 7th June, 1912. 23 pp.
- ECKLES, C. H. and SHAW, R. H. (1913). Variations in the composition and properties of milk from the individual cow. Washington: Government Printing Office. *U.S. Dept. Agricult., Bur. Anim. Industry*. Bull. 157. 27 pp.
- ECKLES, C. H. and SHAW, R. H. (1913). The influence of the stage of Lactation on the composition and properties of milk. Washington: Government Printing Office. *U.S. Dept. Agricult., Bur. Anim. Industry*. Bull. 155. 88 pp.
- ECKLES, C. H. and SHAW, R. H. (1913). The influence of breed and individuality on the composition and properties of milk. Washington: Government Printing Office. *U.S. Dept. of Agriculture, Bur. Anim. Industry*. Bull. 156. 27 pp.
- GORINI, C. (1912). Sur la manière de se comporter des bactéries productrices d'acide et de présure (acido-protéolytiques) du fromage vis-à-vis des températures basses, et leur intervention dans la maturation des fromages. Liège: Joseph van In et Cie, Imprimeurs-éditeurs. Grand'place, 38. (Belgique.) Reprinted from *Revue générale du Lait*, IX. 8 pp.

- GREIG, E. D. W. (1912). Epidemic Dropsy in Calcutta. (Final Report.) *Scientific Mems. Officers Med. & Sanit. Depts, Gov't of India*, N.S., No. 49. 79 pp., five plates. Price 2/6. Calcutta: Superintendent Government Printing, India.
- HINDHEDE, M. (1912). Untersuchungen über den Einfluss einiger Nahrungsmittel auf die Löslichkeit der Harnsäure. Parts I and II. *Skandinav. Archiv für Physiologie*, xxvi, pp. 384-406; xxvii, 87-89.
- HINDHEDE, M. (1912). Untersuchungen über die Verdaulichkeit der Kartoffeln. *Zeitschr. f. physikalisch. u. diätetisch. Therapie*. Bd. xvi, 16 pp.
- LENNEP, D. P. ROSS VAN (VI. 1912). L'influence des substances fixes sur l'anaérobiose dans les milieux de culture liquides. *Folia Microbiologica*, I. Jahrg., Heft 3, 11 pp.
- MCCAY, D. (1911). Investigations into the Jail Diets of the United Provinces, with some observations on the influence of Dietary on the physical development and well-being of the people of the United Provinces. *Scientific Mems. Officers Med. & Sanit. Depts of Gov't of India*, N.S., No. 48. 200 pp. Price 3/-.
- REESER, H. E. (VI. 1912). Complement fixation of different Sera prepared at the State Serum Institute, Rotterdam. *Folia Microbiologica*, I. Jahrg., Heft 3, 23 pp.
- RUCKER, W. C. (1912). Whooping Cough: Its Nature and Prevention. Washington, D.C.: Government Printing Office. Reprinted from *U.S. Public Health Reports*, No. 100, 6 pp.
- SEMPLE, Sir D. (II. 1912). The Vaccine Treatment of Typhoid Fever. *Journ. Vaccine Therapy*, 16 pp.
- SHAW, R. H. (1912). A new method for determining fat and salt in butter, especially adapted for use in Creameries. Washington, D.C.: *U.S. Dept. Agricult., Bur. Anim. Industry*. Circ. 202. 8 pp.
- SÖHNGEN, N. L. (VI. 1912). Ueber Fettsplaltende Mikroben und deren Einfluss auf Molkereiprodukte und Margarine. *Folia Microbiologica*, Jahrg. I, Heft 3, 44 pp., 10 pls.
- Studies from the Department of Pathology of the College of Physicians and Surgeons, Columbia University, N.Y., for the Collegiate Years 1909-1911.* Vol. xii. (1912 Reprints.)
- Studies from the Rockefeller Institute for Medical Research, New York (1912).* Collected reprints vol. xiv. (54 papers).
- TRASK, J. W. (1912). Smallpox in the United States. Prevalence and Geographic distribution during the Calendar Year 1911. Washington: Government Printing Office, U.S.A. Reprint from *U.S. Public Health Reports*, No. 97. 5 pp.
- VIETOR, E. J. F. (VI. 1912). Ueber die proteolytische und antiproteolytische, resp. antitryptische Wirkung des menschlichen Blutserums. *Folia Microbiologica*, Jahrg. I, Heft 3. (Phoenixstraat 18, Delft, Holland.) 59 pp.
- YEARSLLEY, M. (1912). The causes leading to Educational Deafness in Children, with special reference to prevention. London: P. S. King & Son, Orchard House, Westminster. Reprinted from *The Lancet*, vii. 1912. 37 pp. Price 1/-.

THE PRESENT STATE OF OUR KNOWLEDGE OF HAY-FEVER.

(BEING A PAPER PRESENTED AT THE BERLIN CONGRESS
OF THE ROYAL INSTITUTE OF PUBLIC HEALTH.)

BY PROF. W. P. DUNBAR, M.D.

(*Director of the State Hygienic Institute, Hamburg.*)

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Introduction.

IN 1902 the investigations on hay-fever undertaken by me many years previously were brought to a temporary conclusion and their results published¹. I advanced the theory that hay-fever is a disease caused by vegetable poisons contained in the pollen of certain plants. These substances were connected with the proteid of the pollen grain and of a highly specific character. It was thus possible to decide by means of the isolated poisonous pollen proteid, whether a given disease is identical with hay-fever or not. In 1903² I asserted more forcibly that

¹ Dunbar, *Zur Ursache u. spezif. Heilung d. Heufiebers*, 1903. Verlag Oldenbourg, München.

² Dunbar, "Zur Frage betreffend die Ätiologie u. spezif. Therapie des Heufiebers." *Berliner klin. Wochenschr.* 1903, Nos. 24-26.

it was possible to obtain a specific antidote by inoculating pollen proteid into animals, *e.g.*, rabbits or horses. With such an antidote it would be possible (1) to neutralise the pollen poison *in vitro* so that it would no more produce morbid symptoms in hay-fever patients. Further it would be possible (2) by this specific antidote to cure symptoms of the disease already developed. By the timely application of the antidote it would (3) be possible to prevent the onset of hay-fever symptoms.

I was subsequently¹ able to show that by suitable application of the antitoxin hay-fever patients could be relieved from their predisposition, could be immunised to such an extent that they could do without the use of the antitoxin or any other remedies and yet remain free from hay-fever.

Whereas Th. Albrecht, Secretary of the German Hay-fever Association, regards my first publication as a turning point in the history of hay-fever, other colleagues have been less kind in their judgement, and some declare that I have not discovered anything new. Likewise the verdict of laymen, especially of patients, alternates between the two extremes. Some are most grateful, whilst others consider my specific treatment useless. In view of these discrepancies of opinion I shall allow myself to revert to the developments in our knowledge during the last ten years to see which of my original assertions have proved correct and which incorrect.

Historical. .

Whether we are justified in regarding hay-fever as a product of our modern civilisation I am inclined to doubt even more now than 10 years ago. In this period much has been published both in the medical journals and in the newspapers—the latter of course, so far as it dealt with my work, without my initiation and against my wish. Yet in spite of these numerous publications there are still many hay-fever patients who are completely unaware of the nature of their disease. Even in Hamburg on the occasion of a scientific exhibition where my hay-fever investigations were demonstrated, several grown-up residents asked me to determine whether they were hay-fever patients. What astonished me even more was to learn again and again that there are still physicians who deny, or are ignorant of, the existence of hay-fever. In view of this slowness of apprehension, a very general characteristic

¹ Dunbar, "Zur Ursache u. spezif. Heilung d. Heufiebers," 11. *Deutsche med. Wochenschr.* 1911, No. 13.

of the human race, it would appear risky to assert that there could have been no hay-fever patients 500 years ago simply because no case histories have been preserved from those times. It is quite possible that the disease was fairly prevalent even then but that the scientist was wanting to observe the seasonal incidence of the disease, and to bring this to the notice of the public. The oldest convincing case history on record was given by Benningerus in the year 1673¹. He described the case of a lady who suffered from coryza for several weeks in every year at the time of flowering of the roses. A whole century elapsed before hay-fever was next mentioned by an English physician Heberden. But only in the year 1819 the accurate clinical description of the disease was given by a second English physician, John Bostock, himself a subject of this disease. The description given by Bostock was so complete and accurate that very little cause has since been found to alter or to add to it. It is true Bostock only knew the European type of hay-fever, not the autumn catarrh of America and the other types of the disease to which I shall refer presently.

Symptoms of hay-fever.

When the time of suffering approaches, the patients begin to experience from time to time itching of the inner canthus of the eye and the earuncula. This itching sets in, disappears and need not recur for several days. Then it is usually more severe, and one observes marked congestion of the earuncula and perhaps of the adjacent portions of the conjunctival membrane. This is followed by occasional, mild sneezing attacks. Suddenly the disease becomes much more severe. During a walk in warm sunny weather, the patient is attacked by convulsive sneezing fits which hardly give him time to breathe. The eyes itch intolerably, the conjunctiva becomes fiery red and oedematous. Nasal respiration becomes obstructed, the mucous membrane of the mouth and palate tickle unbearably, and this sensation proceeds through the Eustachian tube into the tympanic cavity. This attack is followed by a condition of weakness and enervation which may be so severe that the patient is unable to sit upright in his chair; at the same time the loss of all energy produces a profound mental effect. In many patients the symptoms are intensified by asthmatic attacks which prevent their

¹ With regard to the following older publications, the reader is referred to Sticker's paper "Der Bostock'sche Sommerkatarrh (das sogenannte Heufieber)" in H. Nothnagel's *Spezielle Pathologie und Therapie* (Vienna, 1896) and to my first publication (see p. 105).

finding any peace even at night. Lastly, many patients suffer from intolerable pruritus.

The most peculiar feature of the disease is that on certain days all symptoms disappear, the patient appears to be completely cured, and almost immediately afterwards is re-attacked most severely by all the symptoms described. This enigmatic condition of alternating disease and health lasts as a rule for six to eight weeks, after which period the symptoms gradually decline. The attacks are milder and less frequent, and resemble those occurring at the commencement of the disease; ultimately they quite disappear. But in the ensuing year they recur punctually, and whoever has once suffered from hay-fever as a rule remains its victim all his life.

The disease has always been called "hay-fever," because the patients remarked the regular incidence of the disease with the hay-making season and because they had the sensation of suffering from a febrile disease. Yet thermometric observation hardly ever shows an increase in the body temperature.

In the United States, the same type of hay-fever occurs, but it is less frequent than a similar type of the disease which occurs with the same symptoms in the autumn and also lasts about six weeks.

The etiology of hay-fever.

As is shown by this description, Bostock has taught us to recognise a well defined disease. Yet a long time elapsed before this knowledge was generally accepted by medical men. Even nowadays hay-fever is hardly mentioned in the clinical instruction of our students, and many doctors must admit that they have only by chance heard of the existence of the disease. This is remarkable in view of the fact that hay-fever is one of the, if not *the* most common disease. It is true that it is not one of the scourges endangering human life. But hay-fever is a most troublesome condition, and many patients are so worn out by it every year that they lose all pleasure in life and only yearn for the end of their life of suffering. As soon as the period of disease is over, all is soon forgotten. The patient is doubly happy with the reawakening of a feeling of health and it is only when the next period approaches that he begins to feel anxious and worried. For this reason hay-fever is comparable to sea-sickness; which also has terrors only for him who has once suffered and is forced to undertake a fresh sea voyage. Such sufferers share the lot of the hay-fever patient, that clinical medicine

gives them hardly any serious consideration, whilst they are an incomparable field of operation for pharmaceutical industry which every year brings forth fresh remedies for both diseases, stated to be infallible, yet none of which really helps.

One of the chief reasons why doctors have shown such scanty interest in hay-fever is perhaps to be found in the general view that the majority of the patients are nervous persons, easily influenced by suggestion, or, in other words, that the disease is not a real but only an imaginary one.

Bostock thought otherwise. He believed in an external irritating cause which he sought in moist heat, intense light, and dust. Yet all these stimuli are not characteristic for the period of hay-fever. Elliotson was the first to suspect the *pollen*, and in 1873 Blackley lent further weight to this supposition by very ingenious experiments. He collected the pollen on glass slides coated with glycerine, and was thus able to prove that the pollen is carried through the air for great distances and that particularly in the hay-fever season it is present in great quantity in the atmosphere. He was able to produce asthma-like attacks in hay-fever patients by inhalation of pollen. By rubbing pollen into the scarified skin of the fore-arm and leg he induced erythema. Blackley found many believers in his hypothesis in his day but in the course of time his opponents gained the supremacy.

With the commencement of the bacteriological era, scientists were inclined to regard hay-fever as an *infectious* disease. Influenced by Pasteur's publications, Helmholtz was one of the first (1867) to observe peculiar microbes resembling vibriones in the nasal secretion and to regard them as the cause of the disease. Heymann and Matzushita (1901) carried out experiments which led them seriously to doubt the pollen theory. They inclined to the view that hay-fever was an infection produced by bacteria. They thus confirmed the etiological hypothesis of Georg Sticker (1896) who was led by a critical consideration of all previous observations to regard hay-fever as an *infectious disease*. This excellent scientist then stated his belief that the conviction of the parasitic nature of hay-fever expressed by Binz would be decided in favour of, and to the glory of, Helmholtz, as soon as the investigations were undertaken in the right direction. Since then however Sticker¹ has recognised my theory as correct.

A second, still older hypothesis has found numerous supporters up to the year 1902. According to this view, the disease is caused by the

¹ Sticker, 1912, pp. 141, 144, 145, 159.

odorous emanations of plants. Some authors go so far as to characterise these emanations as "fine smelling particles, finely divided evaporating essential oils, or some problematic substances which develop under the influence of intense sunlight at the time of the first flowering, especially of the rye."

Etiological research into the causes of hay-fever was particularly complicated, because several idiosyncrasies were uncritically confused with hay-fever in consequence of insufficient attention being given to the seasonal incidence of this disease. Thus the zoologist Charlton Bastian regularly observed symptoms resembling this illness when he touched certain worms (*Ascaris megalocephala*) which he preserved in spirit in his collection. By chance this scientist's lecture on Ascaridae coincided with the hay-fever season, and it was inferred that Bastian was a hay-fever patient. It has since been found that these worms contain certain substances which produce hay-fever-like symptoms in some persons. Similarly the hyper-sensitiveness of certain persons towards cats, horses and other animals was confused with hay-fever and used as a proof that all such observations and symptoms were purely imaginative, and that they were comparable to the stories that persons had hay-fever symptoms when looking at artificial roses or the picture of a field of corn. Such deductions gradually led to the conviction that hay-fever was nothing but an hysterical manifestation.

It can be considered as established that certain people suffer from hay-fever-like symptoms when coming in contact with cats, mice, dogs or horses. My own investigations have shown me that such diseases are quite independent of hay-fever and represent a special disease. Thus, *e.g.*, in the case of a lady who suffers from hay-fever-like symptoms whenever she touches cats, I found experimentally a very marked idiosyncrasy to cat's saliva. I shall recur to this question later on (see p. 127) and would now only state that such observations open up a new and probably extensive field of research. I should be very grateful if such patients were referred to me whose illness falls under this category. Their number probably is not very large.

Many of the stories which led to the view that hay-fever was a psychological ailment have been thoroughly disposed of by Georg Sticker (1896), so far as that was possible with the knowledge then available. From the publications appearing since that time one might suppose that the bacterial hypothesis of hay-fever had again become predominant. Yet all hay-fever patients who lived through those times

received a very different impression. They suffered under the general assumption that only *hysterically predisposed persons were subject to hay-fever*. They therefore hardly dared to mention their ailment. They retired from their acquaintances as soon as the hay-fever period approached, for they feared to be looked upon as imaginary invalids and thus to lose the right of being considered as normal, sensible individuals. The majority of physicians at that time still looked upon hay-fever as a nervous disease due to suggestion. Several authors asserted it to be the result of a disorder of the *sympathetic* nervous system, whilst the hypothesis most generally believed attributed it to psychical trouble. Thus hay-fever patients suffered ridicule from their friends and even their physicians, and it was even seriously considered whether they ought not to be excluded from the world and confined in asylums! In 1902 J. Rudolph published his view that hay-fever was a degenerative psychosis. He distinguished a hysteroid and an epileptoid form of spontaneous hay-fever. According to him there could be no doubt that it belonged to the realm of psychopathology, and treatment would thus have to proceed from the psychical side.

My experiments on the etiology of hay-fever.

No one who takes the trouble to study the literature on the causes of hay-fever up to 10 years ago will obtain the impression that up to that time any author had expressed a clear and correct view as to the nature of this disease.

As a very sensitive hay-fever patient I had for a number of years been in a position to investigate the different hypotheses as to the causation of hay-fever. Passing over the theory of local neurosis, of emanations and odours, and the bacterial theory, I gained the firm conviction that only the pollen theory could be correct. A detailed description of the observations which impressed this view upon me will be found in my hay-fever monograph quoted previously. The decisive experiment however could not be performed for several years owing to the difficulty of obtaining *pure pollen*. To-day this fact appears almost incredible in view of the exceedingly simple technique which I afterwards elaborated. Therefore I would at least mention that for several years I consulted different botanical experts as to the best way of collecting a large quantity of pollen. Various methods were suggested, *e.g.*, to lay out large sheets on meadows. My own idea was to aspirate

large volumes of air through bottles, etc. None of these methods was successful. At last I thought of the simple and most natural plan, viz. of shaking flowering ears of corn, *e.g.* wheat, and to collect the pollen falling from them. Still better results attended my subsequent plan of cutting the stalks just before the beginning of flowering and of placing them in water in a warm room. It was thus possible to collect any amount of pollen rapidly, and what was most important to obtain the different kinds of pollen absolutely separate and free from all impurities, even microbes.

Having thus collected pollen of wheat, rye and ray-grass (*Lolium perenne*) the most important question was at once decided. The application of a barely visible trace of pollen to my conjunctival or nasal mucous membrane almost instantaneously produced most intense symptoms of hay-fever. The same experiment had a negative result in a laboratory attendant who was not a hay-fever patient. Within a few days these observations received further confirmation by experiments on two of my assistants who happened to be hay-fever patients and several others not thus predisposed. The results were entirely convincing. The hay-fever patients reacted in the same manner as myself, the remainder proved completely refractory to the administration of pollen. These experiments were afterwards performed with uniform results on a large number of hay-fever patients and normal individuals.

The next question of importance was whether this poison was active *outside of the hay-fever season*. Formerly the pollen theory had been attacked by the argument that the same pollen which were active during the hay-fever season were inactive afterwards. Thus, *e.g.*, Sticker believed Woodward to have demonstrated pollen to be inactive outside of the hay-fever season. He therefore inferred that besides the personal susceptibility the hay-fever attack depended upon the *critical season*. The nature of this critical season was understood by other authors in the light of an internal change, a kind of spring evolution, taking place in the patients at this time of the year. This view did not appear to me to be justified, since this internal change would have to take place once a year in European patients in the spring, whereas in some of the American patients who suffer in spring and autumn the change would occur twice a year.

My experiments had the following result. Pollen which were carefully dried immediately after collection always proved active. But pollen placed in glass-stoppered vessels directly after collection underwent decomposition which was rendered obvious by liquefaction. Such

pollen in which the poisonous proteid was decomposed by the action of the enzymes always present in the pollen grain have since repeatedly been found inactive. This observation by the way affords a simple explanation of the occasional occurrence of hay-fever attacks in winter. Pollen which settle in a dry locality may retain their activity through the winter, and even for many years. In a flower which had been preserved for 11 years in a collection an unimpaired action was demonstrated. On the other hand, in pollen settling in the open the poisonous ingredient is destroyed by the first rainfall. Since pollen is carried down from the air by rain, a simple explanation is afforded for the hitherto inexplicable cessation of the hay-fever attacks on certain days. These days have been definitely proved to be the rainy days.

The grass pollen thus isolated had therefore served to fulfil the chief postulates of etiological proof which I had laid down at the beginning of my investigations. The supposed etiological agent, if *free from any contamination*, must cause hay-fever in the susceptible person at *any season of the year*, but must be absolutely *inactive towards normal persons*. This was not however the end of the question.

The grass pollen granules are so small as to be hardly recognisable with the naked eye, yet both in their *physical structure* and their chemical constitution they are very complex. Many pollen grains are armed with sharp prickles. Partizans of the pollen theory previously believed these prickles to be the cause of hay-fever. They supposed the hay-fever patients to be extremely sensitive individuals whose mucous membranes react intensely to a mechanical stimulus which has no effect upon the mucous membranes of normal persons. As it happens, some of the pollen grains which were formerly regarded as the principal causes of the disease have a rough or prickly surface. Besides, the earlier supporters of the pollen theory thought chiefly of the pollen of such plants whose flowers have a penetrating odour. The disorder was therefore frequently designated not as hay-fever, but as rose flower fever, lime flower fever, etc. These views were at once discarded when I demonstrated that the pollen of most of the plants which I found active have an absolutely smooth surface. This is true of all species of Graminaceae, 32 of which I investigated. The grass flower, too, has no scent.

The flowering period of the lime and rose usually coincides with that of the grasses. But in 1902 I was able decisively to disprove the obstinate belief of many hay-fever patients in the importance of the

lime flower. For in this particular year the flowering of the limes had been delayed in our neighbourhood for three to seven weeks, whilst the grasses flowered at the usual time. The chief period of illness in our patients was over when the lime flower attained its maximum, and thus for once they were able to enjoy with impunity this scent which in other years had filled them with dismay.

Likewise in 1912, when the first half year showed quite abnormal climatic conditions, the lime did not begin to flower until several weeks after the grass, *i.e.* at a time when the acme of hay-fever had passed. G. Sticker¹ still lays stress upon the etiological importance of the lime flower for certain patients and supports this with the assertion that the number of my experiments is still insufficient.

On the contrary I must point out most emphatically that in not one case has it been demonstrated that hay-fever patients can be influenced by lime flowers, although since my first publication I have constantly experimented in this direction and have since administered lime pollen to a number of hay-fever patients with absolutely negative effect.

Still, I can understand the obstinacy with which patients believe in the causation of hay-fever by the scent of roses and limes. For many years I myself banished every rose and every other scented flower from my rooms during the hay-fever season and was convinced of having thus reduced my sufferings. This result was however due to my having simultaneously kept my windows scrupulously closed.

Almost simultaneously with the grasses, the pines (*Pinus silvestris*) begin to flower. They too are anemophilous and disperse so large an amount of yellow pollen that it occasionally fills the air with thick clouds. In such cases one speaks of "sulphur rain." Against the energetic opposition of several patients I was able to prove this pollen also to be innocuous.

A number of similar and varied experiments showed with certainty, in opposition to Blackley's assumption, that only *certain, specific pollen cause hay-fever*, whilst other pollen, even if armed with sharp spikes, are harmless.

We will now consider *emanations, scents, essential oils*, etc. If a vessel containing considerable quantities of grass pollen is opened, a honey-like odour is noticed. This is devoid of any influence upon hay-fever patients. The harmlessness of rose-scent and the still more frequently incriminated lime-scent has been repeatedly proved by

¹ Sticker, *Das Heufieber und verwandte Störungen*, 1912, p. 21.

me in an extensive series of experiments. There remained only to determine the effect of *essential oils*. The oily and waxy constituents of pollen when applied to the eye or nose of hay-fever patients in small quantities produced a burning sensation. This was however quite different from the peculiar nettlerash-like itching which every hay-fever patient knows and which can hardly be confused with any other sensation. What was even more important, these substances affected normal persons in the same manner. Under ordinary conditions the quantity of such alcohol- and ether-soluble substances coming in contact with our mucous membrane is so slight that the threshold of stimulation is not passed and no such sensations can develop.

The pollen of grasses are distinguishable from those of many other plants by the presence of a great number of small *rods* resembling bacteria. These had already been noticed in 1877 by Patton. He stated that the rods showed energetic movements when they emerged from the pollen grain and inferred that they were the active principle of the pollen, that they penetrated actively into the mucous membrane and the circulation, thus producing the symptoms of hay-fever. For a short time, I too was inclined to attribute an etiological importance to them. I thought that they did not consist of pure starch, but of a mixture of starch and proteid. As soon as I had obtained a sufficient quantity of grass pollen these bodies could be isolated by repeated centrifuging and washing: they were then shown to be inactive towards hay-fever patients.

Certain considerations to which I shall refer later soon led me to believe that the active principle was a proteid. In this view I was confirmed by the observation that the alcoholic precipitate obtained from the salt extract of a small amount of pollen was intensely active upon hay-fever patients but not upon normal persons. Later, when larger quantities of pollen had been collected, my etiological investigations were continued with the isolated proteid of grass pollen. Against this procedure the objection has been raised that I was not working with the genuine poison but with a denaturated poison. This assertion is entirely unfounded. My critics have neglected to furnish any proof of their assumption. It is obviously far more scientific to work with the isolated poison than with the entire pollen or with the unstable pollen extract. This extract I also employed in my earlier investigations, but only until I had determined that the poisonous principle was bound up with the proteid. Subsequently

Dr Kammann¹ at my request sought to determine which portion of the pollen proteid is the active principle. He found this to be the *albumin* fraction, whilst the globulins proved quite harmless to hay-fever patients.

Recently, Kammann has carried the purification of the poison still further. For this new toxin, which will be described in a later publication, the following points are of importance: (1) addition of diastase to remove the starch rods, (2) extraction with sterile distilled water (instead of salt solutions), (3) liberation of the toxin from the ballast of proteid bodies by intracellular fermentative processes, (4) solution in sterile distilled water, with subsequent addition of the necessary amount of salt.

After having proved that the pollen proteid of certain plants was the specific cause of hay-fever, I was enabled still further to elaborate my experiments by placing them on a quantitative foundation. The proteid is easily extracted from pollen by suitable salt solutions, from which it is obtained in pure condition by precipitation with alcohol or by dialysis, and dried. In this condition it retains its efficacy unimpaired for years.

Referring to my first convincing experiments on the etiology of hay-fever, it is only fair to point out that they did not correspond with the natural development of the hay-fever attack. This was more closely imitated by the following experiment. A hay-fever patient and a non-susceptible person (the control) stood in a large glass cupboard in which rye pollen were distributed. The patient developed an attack, the control remained free from symptoms. In this experiment however it was not possible to determine the number of pollen inhaled by the experimenters. The question had not as yet been satisfactorily solved whether during the hay-fever season the number of poisonous pollen present was sufficient to cause an attack. In this direction the experiments of Blackley mentioned previously had paved the way. By the use of a method suggested by Phœbus he had performed accurate counts of the pollen which adhered at different seasons to slides which had been rendered sticky with glycerine. My collaborators, especially Liefmann², found that at the time of the worst hay-fever attacks in the centre of the city of Hamburg no less than 250 grass

¹ O. Kammann, "Zur Kenntnis des Roggenpollens und des darin enthaltenen Heufiebergiftes." *Beitr. z. chem. Phys. u. Path.* 1904, vol. v. p. 346.

² Liefmann, "Ein Beitrag zur Frage nach der ätiologischen Bedeutung gewisser Pflanzenpollenkörner für das Heufieber." *Zeitschr. f. Hygiene u. Infektionskrankheiten*, 1904, vol. XLVII. p. 153.

pollen settled in 24 hours upon a surface of one square centimetre, *i.e.* about 2,500,000 pollen upon a square metre. It was most interesting to note year by year how the first appearance of a few pollen in the air coincided with the time when the patients began to feel the first itching in their eyes; how the suffering increased with the number of pollen in the air; how on rainy days the pollen was absent on the slides although these were protected from the rain by suitable covers. In the beginning of June the grass pollen surpass in number all the other pollen in the air; from the third week of July they gradually disappear, but a few are found to linger until the end of July or even the first part of August. These results would appear to explain satisfactorily the periodic variations and the occurrence of isolated hay-fever attacks.

It was still necessary to gain an accurate estimate of the effect to be expected from a given number of pollen grains. Dr Kammann has found that about 40% of the organic substance of the grass pollen consists of proteids. He also found by enumeration that about 20 millions of rye pollen grains weigh one gramme. From these figures the toxicity of a single pollen grain was calculated. With exactly prepared solutions of the poisonous pollen proteid it was further possible to determine how many pollen grains were required in the different patients to produce mild, medium and severe attacks. We thus found, as was to be expected, considerable differences in the susceptibility of the patients. Normal persons were unaffected even by the instillation of concentrated proteid solutions into the eye or nose. The majority of hay-fever patients were affected by one drop ($=\frac{1}{20}$ to $\frac{1}{30}$ c.c.) of a solution 1:20,000 to 1:30,000. But some patients were found susceptible to one drop of a million fold dilution. This amount corresponds to the contents of two or three pollen granules.

H. Liefmann has constructed an *aeroscope* with which apparatus he endeavoured to find out how many pollen grains were inhaled with one breath during the principal hay-fever season. Near a rye-field he found one breath to carry two or three pollen grains, but even in the centre of a large town 20 to 30 pollen grains were found in a cubic metre of air. Thus the question as to the quantitative conditions in hay-fever has also received a satisfactory solution. From the experiments already described the definite conclusion can be drawn, that *the pollen proteid of certain plants, especially of all the grasses hitherto examined, is the cause of hay-fever.*

In connection with these investigations, I have examined with my collaborators the pollen of 106 other plants—the result being negative

although I tested particularly such pollen which had been stated by others to cause the symptoms of hay-fever. Besides the pollen of the 32 species of Gramineae and Cyperaceae I have only found the following pollen to be toxic: honeysuckle (*Lonicera caprifolium*), Lily of the valley (*Convallaria majalis*), *Polygonatum multiflorum*, *Oenothera biennis*, rape (*Brassica napus*) and spinach (*Spinacea oleraceu*) as well as a number of Composites.

When I was informed that in China hay-fever-like disease is observed at the time of flowering of *Ligustrum vulgare*, I examined its pollen and found them to be toxic. In S. W. Africa similar symptoms are observed when the grasses flower, the half-breed population being particularly affected. A European was forced to leave Africa at this season, his sufferings being intolerable, yet in Europe he remained quite healthy. Examination showed him to be unaffected by grass pollen. At the same time in S. W. Africa the acacias flower, and these have been suspected to be the cause of the disease. Yet this patient was not affected by the pollen of *Acacia dicabata* and of *Robinia pseudacacia*. Uhlemann has since found that S. W. African hay-fever is carried by the pollen of a species of *Eragrostis*. The extract of these pollen which he kindly sent me was tested on persons susceptible to European hay-fever; of these some were not affected, some only showed objective but no subjective symptoms, whilst others were affected both objectively and subjectively. *Eragrostis* belongs to the Gramineae. This would therefore be the first instance where the pollen proteid of a grass did not influence European hay-fever patients. I am at present endeavouring to cultivate *Eragrostis* in Hamburg, in order to amplify the results obtained hitherto.

A disease of paramount importance is the autumnal catarrh which begins in the United States about the first part of September and which also lasts about six or eight weeks. This autumn catarrh is reported to occur far more commonly in the States than the spring form of hay-fever. I have had the opportunity of testing a number of American patients. It was thus found that those patients who only suffer in the autumn are unaffected by the pollen proteid of grasses, but that they always react to that of golden-rod (*Solidago*) and of ragweed (*Ambrosia*). Of these composites a large number of species has been investigated, all being found active. The same patients react also to the pollen proteid of *Chrysanthemum* and Asters.

On the other hand, those American patients who only suffer from the spring form, not from the autumnal catarrh, are susceptible

to the pollen of grasses, but not to those of golden-rod and ragweed.

A third group of patients in America suffer from hay-fever symptoms between the middle of May and the end of November. These unfortunate individuals react both to grass pollen and to the causes of autumnal catarrh. Golden-rod and ragweed belong to the commonest weeds in the United States. They are found not only in every meadow, field, wood- and road-side, but even in the towns they grow in any ill-kept streets and steps. In Europe they are absent. Golden-rod can be brought to flower with us. Its pollen does not disperse nearly so much as those of ragweed. All our endeavours to bring ragweed to flower here were in vain until 1911. In this year, remarkable for its excessive heat and drought, I was successful for the first time.

The results that I have laid before you up to now can be regarded as further important arguments in favour of the specific pollen theory. There still remain several doubtful points requiring elucidation before the whole enigma of the disease is understood. One matter which is of the utmost importance is individual susceptibility.

Individual susceptibility.

The foregoing remarks have shown that at certain seasons everyone—including the inhabitants of large towns—is attacked by numerous pollen grains which settle upon the skin, the conjunctival membrane, which penetrate into the nose in breathing and into the mouth during speech. The great majority of mankind is totally unaffected by this pollen, only a small proportion suffer from the disease. The active pollen proteid is not therefore a poison in the ordinary sense of the word; it is a substance harmless to most human beings, and active only in persons possessing a certain degree of susceptibility. Hay-fever therefore depends upon an *individual predisposition*. This is a condition hardly met with in the case of ordinary poisons of the pharmacopoea. In infectious diseases it is already much more marked. If *e.g.* the cholera or typhoid microbes are disseminated throughout a town by the water supply, it is only a comparatively small proportion of the persons infected which acquire the disease. The explanation is that the cholera microbe—which only becomes fatal in consequence of its proliferation in the human body—does not find the conditions for its life and propagation in most persons. The further fact that only about one half of the cholera patients die of the disease may perhaps be

explained in a similar manner by considerations of quantity. I do not however know a second instance of one substance proving absolutely indifferent to a part of mankind, whilst appearing as one of the most intense poison to others. There is reason to believe therefore that the individual predisposition in hay-fever is something special.

One might assume the hay-fever poison to enter into the circulation in certain persons, viz. the hay-fever patients, but not in others. This no doubt occurs, as I was able to prove by the demonstration of immune substances in the blood, a result to which reference will be made later. It will only be necessary to mention here that such specific substances are only found in hay-fever patients soon after the hay-fever season but have disappeared six months afterwards. On the other hand in the normal persons examined we never found such substances even directly after the hay-fever season. The gradual disappearance of the immune substances from the circulation is readily understood. We know from animal experiments that such substances appear in the circulation at certain intervals after the inoculation, and that they only remain in the blood if the treatment is continued, but gradually disappear after its interruption. At first sight the demonstration of immune substances in the blood of hay-fever patients appeared as a satisfactory explanation of the disposition. But soon doubts arose. For when the investigations were continued, these immune substances were not found in other hay-fever patients; in the following year I was not even able to find them after the hay-fever season in the same patients who had previously given a positive result. It will be shown later on, that specific immune substances were not found even in patients or normal persons who had received several subcutaneous injections.

The following is however a still more serious objection. A colleague susceptible to hay-fever who for many years assisted me in my investigations with the greatest self denial, injected himself subcutaneously in the fore-arm with a solution of grass pollen proteid. Within the next half hour very severe symptoms developed in the mucous membrane of eye, nose and mouth. He suffered from pain in the chest, expectorated a tough mucous sputum and perspired profusely. Respiration was accelerated and difficult, the pulse-rate quickened, the voice hoarse. Fifty minutes later urticaria developed all over the body. The results of the inoculation were still felt by the patient on the next day. At the site of injection considerable tumefaction occurred which remained for five days. The same symptoms have occurred repeatedly

when I injected myself with hay-fever poison. Yet another colleague, not susceptible to the disease who injected the same quantity, reacted only with a slight tumefaction at the site of inoculation. The pollen proteid was not therefore poisonous for him. Many hundreds of animal experiments have again and again proved that the pollen proteid is not a poison in the ordinary sense of the word and that it is harmless even when injected directly into the blood circulation.

The permeability of the skin of hay-fever patients for this poison is different from the normal. Even in individual patients various reactions may be observed. In some patients the application of a drop of pollen proteid solution to the skin is followed within a few minutes by *erythema*. In other patients, who may in other respects be very susceptible, the skin proves refractory to the pollen solution.

Indirectly, these results can serve as a guide for the study of individual predisposition, for they enable us to approach the question whether hay-fever is to be regarded as a reaction of supersensitiveness. Before entering into this question I must deal with the *older attempts to explain the hay-fever predisposition*. This subject has, I believe, received a fairly exhaustive treatment in my monograph¹ up to the year 1902. The details will not therefore require to be repeated here. It will suffice to mention briefly only such views which I have found still to influence several oculists and rhinologists although being in direct contradiction with the result of my experiments. The first point requiring mention is that all statistical enquiries have shown that hay-fever by no means depends upon certain constitutional diseases, *e.g. gout*. In fact only a small percentage of hay-fever patients show this diathesis. Many observers believed hay-fever to depend upon *deviation or impermeability of the upper air passages*. Others assume a local neurosis of the fifth cranial nerve leading to sensitiveness of certain mucous membranes. The incorrectness of such views is shown by my already mentioned experiments. Not only the entire skin of many patients reacts to the poison, but even the subcutaneous injection is followed by characteristic hay-fever attacks. Finally, the fact that the mucous membrane of the anus of hay-fever patients reacts to the pollen proteid, I believe to have conclusively disproved all hypotheses based upon the assumption of a specific sensitiveness of a cranial nerve or a mucous membrane of the head.

Suggestion as has been shown plays an important part in the

¹ Dunbar, *Zur Ursache u. spezif. Heilung des Heufiebers*, 1903. Verlag Oldenbourg, München.

explanations of hay-fever disposition. I can treat it in common with the subject of the effect of *certain odours*, *e.g.* of flowers, cats, horses, etc. In order to obtain some evidence, I undertook the following experiment. A number of hay-fever patients received simultaneously a drop of a colourless and odourless fluid into the eye and the nose. Some reacted, others did not. None knew what had been administered. Then the reverse experiment was performed, and now those patients who had reacted at first showed no irritation and *vice versa*. The one fluid was normal saline, the other a solution of grass pollen proteid. None reacted to the former, all to the latter. Such experiments have been frequently repeated with different modifications and the results have always been consistent. It is therefore impossible to explain hay-fever as due to suggestion or the like.

Hay-fever has been regarded as the result of *higher civilisation*. It is true that very few cases are observed in the labouring classes, and also that the greatest contingent of hay-fever patients is furnished by the Anglo-Saxon race, Germans, Englishmen and Americans. The disease occurs occasionally among the romanic and other peoples. In St Louis I saw a young negro, employed as a lift-boy, who suffered from hay-fever. Among the particularly susceptible Anglo-Saxon race the mental workers are specially affected. It has been stated that men are twice as liable to hay-fever as women, but this is not confirmed by accurate statistics. Frequently the disposition is reported to have followed mental overwork or great excitement, *e.g.* after examinations, in officers after the manœuvres. Very often the disease has been found to be inherited. Most commonly it seems to have occurred during convalescence after a severe attack of influenza. Other severe affections, *e.g.* difficult labour, are definitely stated by some patients to have led to the hay-fever susceptibility.

Is the conclusion justified that hay-fever is the result of damage to the central nervous system? The view formerly held that hay-fever patients were particularly nervous, excitable persons, cannot be upheld in this generalisation. If it is due to any damage of the central nervous system, the effect is only evidenced by the hay-fever disposition. Hundreds of hay-fever patients have informed me that outside of the hay-fever season they are always healthy, and among such patients I have frequently found most phlegmatic natures.

Those *idiosyncrasies* which show some resemblance to hay-fever, *e.g.* the susceptibility of certain persons to strawberries, crabs, to iodine, antipyrine, bromides and quinine salts one is nowadays inclined to

regard as supersensitiveness, *i.e.* as *anaphylactic* symptoms. Ten years ago I considered the possibility of the hay-fever disposition belonging to this class of conditions. E. v. Behring¹, Knorr², and others had already stated that certain animals which had received a sub-lethal dose of diphtheria toxin, subsequently showed excessive reactions with far smaller doses. The supersensitiveness of tuberculous patients to the proteids of the tubercle bacillus, observed by R. Koch³ in 1890, is also one of these phenomena. But at that time the regular occurrence of this phenomenon had not yet been recognised. Since then an almost boundless literature has developed with regard to this subject. It is safe to say that at the present time the anaphylactic processes are predominant in all research on experimental therapeutics. It is characteristic of anaphylaxis that in no case has the occurrence of anti-bodies been observed to the *anaphylactic toxin* which was shown by Friedberger to be the cause of these symptoms. A further important observation is that this supersensitive condition can be transferred by injection of the blood serum of anaphylactic animals or human beings (passive anaphylaxis). Lastly, that after recovery from the anaphylactic shock an anti-anaphylactic condition develops. Any attempt to regard the hay-fever disposition as an anaphylactic condition must therefore take into account these three facts.

As regards *passive anaphylaxis*, I have injected the serum of hay-fever patients intravenously into guinea-pigs. Twenty-four hours later this was followed by an intravenous injection of rye pollen proteid. Both in these cases and even where I injected the serum of guinea-pigs, which had received repeated injections of pollen proteid, into other guinea-pigs, and followed this by injection of pollen proteid, I only noted mild, transient convulsions and a fall of their temperature to 36.1° C. These results are not in favour of the anaphylactic hypothesis.

The hay-fever patient himself does not become *anti-anaphylactic* after a hay-fever attack. The decline of hay-fever on certain days is more easily explained, as we have seen, by a reduction in the quantity of pollen as it coincides with the rainy days. All observers appear to be agreed that the hay-fever patient does not become less, but rather *more* sensitive after an attack.

One of my collaborators has stated that he had experimentally

¹ v. Behring and Kitashima, *Berliner klin. Wochenschr.* 1911.

² Knorr, *Habilitationsschrift*, Marburg, 1895.

³ Koch, "Weitere Mitteilung über ein Heilmittel gegen Tuberkulose." *Deutsche med. Wochenschr.* 1890.

applied pollen proteid to his eye thousands of times, yet the minimum dose was not altered. I have observed the like hundreds of times.

All clinical observations agree that in hay-fever anti-anaphylaxis does not occur, and that therefore the hay-fever disposition is not in this respect comparable with anaphylaxis.

In the third point also hay-fever does not coincide with our present definition of anaphylaxis, for it is possible, as will be shown subsequently, to prepare a true antitoxin against pollen proteid.

On the other hand all my experiments and considerations are opposed to the view that the hay-fever disposition is identical with anaphylaxis. They are rather explicable by an opposite hypothesis.

Other considerations must also be borne in mind. The anaphylactic hypothesis is based on the following supposition: In some way the hay-fever patient receives into his body a considerable amount of pollen proteid. He would thus become *sensitised*, i.e. rendered sensitive to the subsequent injection of pollen proteid. Yet I have repeatedly observed that persons living in Germany who have never been in America and thus never came into contact with the pollen of golden-rod or ragweed, developed hay-fever at the first contact with such pollen proteid. On the other hand, I found that *normal persons never develop hay-fever after the subcutaneous injection of grass pollen proteid*, although the dose injected was a multiple of what these persons could have absorbed in a natural manner in the course of many years. Previous to the hay-fever season of 1912 I injected a normal individual at intervals of five days with such quantities of hay-fever poison as they could never have absorbed under ordinary conditions, quantities which in the hay-fever patient would have led to alarming symptoms. Although this person daily took long walks through meadows in full flower during the hay-fever period, he has not shown the slightest signs of the disease.

Besides, the results of the injection of the toxin in hay-fever patients to which I shall refer later, are decidedly opposed to the view that hay-fever could be an anaphylactic condition.

Yet even if we should be able to characterise the hay-fever pre-disposition as a sensitisation comparable to anaphylaxis, we should not have obtained a satisfactory explanation of the individual susceptibility. For we should still have no explanation of the fact that normal persons cannot be rendered susceptible even by the subcutaneous injection of pollen proteid. *The normal person has not therefore the capacity of reacting to this proteid*, whilst the hay-fever patient is endowed with

it to a considerable degree. We must patiently proceed with experimental research and endeavour somehow to obtain a key to the explanation of these phenomena.

Further experiments to elucidate the hay-fever disposition appear to be called for because this is the only disease with which we can safely experiment on human beings, and because the results thus obtained can at once be used in the explanation of other toxic and infective diseases.

A year ago I showed that during the hay-fever period the serum of hay-fever patients has a different influence upon erythrocytes to that of normal persons. It haemolysed *e.g.* the red blood corpuscles of guinea-pigs, rabbits and sheep. Six months later the serum of the same patients had quite or almost lost its haemolytic effect upon these blood corpuscles, but where haemolysis did not occur, we observed agglutination. These experiments were continued by Dr Gaechtgens with monkeys. He found that the serum of a monkey before the injection of pollen proteid did not influence the erythrocytes of rabbits and guinea-pigs and only slightly agglutinated those of sheep. Ten days after the injection of pollen proteid the red blood corpuscles of rabbits were energetically agglutinated, those of guinea-pigs slightly haemolysed, whilst those of sheep were not influenced. A control monkey injected with horse serum however showed a similar reaction. Thus the path which we had hoped to find again seemed lost. I attach the greatest value to the further continuation of these experiments.

In this connection I may mention a series of experiments which were also begun several years ago. They were based upon the observations of Preston Kyes that cobra venom is activated by lecithin so as to dissolve red blood corpuscles to which it otherwise proved inactive. Similar experiments were undertaken in this direction with pollen proteid, at first without any valuable result. Dr O. Kammann has since been investigating these phenomena with the following results:

If (1) washed blood corpuscles are treated with pollen toxin, they are not affected. The same is the case if (2) the corpuscles are treated first with *lecithin* and then with pollen toxin. But if (3) pollen toxin is mixed with washed ox blood corpuscles, and lecithin added about ten minutes later, haemolysis results. The same result is obtained if (4) the blood corpuscles are treated with pollen toxin, then separated from the fluid and taken up in a lecithin solution. These experiments will shortly be published in detail. Possibly they have a bearing upon the questions considered previously. It is certainly worth while to

enquire whether the individual susceptibility of the hay-fever patient can be due to the presence of greater quantities of lecithin in their blood than are in the blood of normal persons. Proceeding from the idea which I firmly believe in, that hay-fever is due to damage of the central nervous system, it is possible to advance one step further and to assume that such damage might be due to an excessive secretion of lecithin into the blood. The interesting discovery has recently been made by Dr Kammann that the pollen toxin contains a *lipolytic* ferment; this has suggested to him that possibly this secretion of lecithin might be referable to the pollen toxin. Still, in all such hypotheses we are brought back to the postulate that normal persons ought also to be influenced by solutions thus prepared. All investigations undertaken in this direction have hitherto had no result. Therefore even these most recent experiments do not afford sufficient reason for me to depart from my view that we cannot at present explain the enigma of hay-fever disposition.

In this connection it will be worth while to refer to the *susceptibility of certain persons to horses, cats and other animals*. I have mentioned that several genuine hay-fever patients were found to suffer from similar symptoms outside of the hay-fever season, as soon as they entered a stable or circus, or only rode in a horse-drawn vehicle. According to one author who looks upon hay-fever as an anaphylactic condition the patients are so highly sensitised towards horse serum, that the merest trace of skin excretion would suffice to produce an anaphylactic attack which may resemble an attack of hay-fever. In order to put this view to the test I undertook the following experiment. A lady who was affected with hay-fever symptoms whenever she entered a stable or circus or drove in a carriage was unaffected by the application of scales removed from horses with the curricomb. This lady was especially suited for such experiments, because she showed no trace of nervous or hysteric tendency. Acting upon my suggestion she visited a horse show after having treated her eyes and nose with pollen antitoxin derived from a horse. Although she was present for hours at the show, not the slightest symptom of irritation was noticed. In this, as in several other cases, I was able therefore to prevent the irritation caused by horses by means of a preparation rich in horse proteid. This critical experiment will have to be considered in future by authors desirous of propounding new hypotheses for the idiocyncrasies described.

The same patient stated that she was attacked by hay-fever as soon

as she touched a cat. The following experiments confirmed her statement. I let her stroke the fur of a cat a few times and then touch her cheek with her hand. Neither hand nor eye showed any symptoms. The eye—which had been kept closed while she touched her cheek—remained unaffected for ten minutes. I was prepared to disbelieve her statement, but the lady definitely affirmed that the attack would come. After 15 minutes she felt itching in the eye corresponding to the cheek she had touched. At the same time the conjunctiva of this eye began to be congested. Within the next few minutes congestion increased, the caruncula became dark red, the itching and burning became worse. The patient asserted that if we allowed the attack to proceed it would develop into a severe hay-fever attack. As soon as pollen antitoxin was administered to the eye, the subjective symptoms were improved, and soon after the congestion declined.

Hairs cut from the same cat were extracted with ether, alcohol and normal saline solution. These extracts proved inactive. I then asked my patient to touch the hairs after they had been kept for 24 hours, also without any effect. But when she touched hairs that had been cut off five minutes before a positive result was obtained. Other substances had meanwhile been examined, but all were found to be inactive, although I had throughout endeavoured to produce an effect by means of suggestion.

Since it was possible that traces of saliva adhering to the hairs might contain the active principle, I collected some saliva by allowing the cat to bite upon a cotton wool plug saturated with milk. After touching this plug the lady had a typical attack. I am therefore convinced that we are dealing here with a very marked specific idiosyncrasy against cat's saliva. Further experiments in this direction are being conducted. The case appears particularly important to me because such idiosyncrasies to my mind are very nearly related to hay-fever, for otherwise it would be impossible to influence them favourably by pollen antitoxin.

It will be evident from the foregoing remarks that the problem of individual hay-fever susceptibility has not yet received a satisfactory solution. It is far simpler to decide in any given case whether the disease is hay-fever or not.

The observations already described regarding the irritative action of the pollen proteids of certain plants upon the mucous membranes of hay-fever patients have been employed from the first for purposes of diagnosis.

The diagnosis of hay-fever.

It has been stated that a hay-fever patient reacts with symptoms of the disease after the instillation of a drop of 1 in 20,000 pollen proteid solution, whilst normal persons are not affected by solutions containing one per cent. or even more of the proteid. This is a simple, certain and conclusive means of diagnosing hay-fever, a test the like of which cannot be applied to any other disease.

By this test it is even possible to determine whether the patient is subject to the European or the American autumn fever, or both forms. It is thus possible to decide absolutely whether the patient is the victim of nervous coryza or other symptoms resembling hay-fever. We can ascertain whether persons who believe themselves to be sensitive to the odour of cats, horses, etc., judge their affections correctly or are subject to genuine hay-fever.

Much confusion as to the inefficiency or uselessness of hay-fever remedies would be avoided if the physician regularly controlled his diagnosis in a scientific manner in every case suspected to be hay-fever. Messrs Schimmel & Co. in Miltitz have readily undertaken to furnish any physician with the toxin free of charge. It is supplied weighed out for use in a very convenient outfit and is called the "hay-fever diagnostic."

In this connection I may be permitted to remark that the whole method of conjunctival diagnosis or *ophthalmo-reaction* which is so commonly practised nowadays is the outcome of my hay-fever reaction, although it is generally associated with other names. Ten years ago it was also shown by my investigations that the hay-fever poison produces irritation in some patients when applied to the uninjured cutis.

Experiments to obtain specific immune substances, particularly an antitoxin.

The experiments already described will have proved that the specific hay-fever poison is identical with or at least inseparable from a proteid.

Whether there was any chance of obtaining a specific antitoxin was doubtful at the beginning of our experiments. Behring¹ had, it is true, shown the possibility for tetanus and diphtheria, of obtaining specific antitoxins by the use of the specific bacterial toxins. Ehrlich² too had prepared antitoxins to the toxic proteids derived from certain higher plants (abrin, ricin). The pollen proteid of grasses is not a poison comparable to those I have mentioned, since it is inactive towards most men and animals. It is toxic only for a small fraction of human

¹ Behring and Kitasato, "Über das Zustandekommen der Diphtherieimmunität und der Tetanusimmunität bei Tieren." *Deutsche med. Wochenschr.* 1890, No. 49.

² Ehrlich, "Experimentelle Untersuchungen über Immunität." *Ibid.* 1891, Nos. 32 and 44.

beings. Further, it has been shown by animal experiment that it does not belong to proteids of high avidity: thus *e.g.* the proteids of fish-roe when injected into animals lead to a far more energetic formation of anti-bodies (precipitins, cytolsins) than the pollen proteid, and are thus shown to possess a far higher degree of avidity.

Although the conditions did not appear favourable, my first experiments with rabbits in the spring of 1902 led to unexpectedly promising results. The serum of *rabbits* which had received several intravenous injections of pollen proteid (1) *in vitro* influenced and altered the proteid in such manner as to render it inactive to hay-fever patients. It was also possible (2) by the application of this immune serum promptly to remove the irritation induced in hay-fever patients by the pollen toxin. Finally (3) it was proved that instillation of the poison into the conjunctiva had no result, if the immune serum had been instilled previously. This success encouraged me to undertake inoculation experiments with larger animals, viz. with goats and horses. *Goats* almost always proved refractory to the pollen proteid, and even after prolonged treatment most of them produced no antitoxin. Only one of them fainted after every injection and finally died immediately after an injection in consequence of its supersensitiveness, or, as we should now call it, from anaphylactic shock. Such occurrences I now believe to be preventable, since we have given up injecting the crude pollen extract, and inject the pollen proteid after removal of all the non-specific ballast (Kammann). *Horses* also showed very varying reactions to the pollen toxin. I am not sure whether it was only a chance that among the comparatively large number of horses which I inoculated, common farm horses proved refractory, whilst well-bred animals, particularly broken-down racers, reacted severely. It seemed quite possible that the exciting work required from such animals might have rendered them susceptible to the poison just as we imagine civilisation to have affected man. This can only be decided by an extensive series of experiments. I would note however that it was our constant experience that horses which reacted to pollen toxin always did so at the first inoculation. Further injections did *not increase their susceptibility*; on the contrary they *tolerated considerably greater quantities of the poison at a later stage of treatment*. Thus the initial dose often caused symptoms of such severity that the consulting veterinary surgeon expected the animal to succumb. Yet in the course of treatment they received as much as 30 times the original dose without showing any symptoms. Apart from fever, profuse perspiration,

convulsive trembling and loss of appetite, the most noticeable feature was the formation of urticaria-like wheals. The oedematous swelling at the site of injection in some horses had a diameter of 50 to 75 centimetres. In other cases the first injection was followed by an eruption of wheals the size of a walnut all over the body of the animal; subsequent injections of larger quantities of the toxin were tolerated without the formation of such eruptions.

A somewhat inaccurate critic has brought a good deal of confusion into the hypotheses of hay-fever by the assertion that the horses become more susceptible after the injection, and later on react to smaller doses, but do not tolerate increased amounts. This assertion disagrees with all our observations. All hypotheses and conclusions founded on it are therefore baseless. One of these fallacious conclusions was that it would be impossible to prepare a specific antitoxin for pollen toxin. In some instances it is true highly immunised horses are reported to have shown congestion of the conjunctiva after instillation of the pollen proteid. I have never witnessed such occurrences, but their possibility must be admitted in view of the observation by Walther¹ of hay-fever symptoms in a horse.

Certain rabbits and horses therefore are exceptionally well suited for the preparation of a specific pollen antidote, or antitoxin. The *existence* and *potency* of such antidote can only as yet with certainty be shown by clinical methods, or by combined examination *in vitro* and in the body. *Precipitins* I have never observed except in the serum of hay-fever patients where a very weak though unmistakable reaction was noted. In numerous rabbits no trace of precipitins could be demonstrated in spite of numerous injections of pollen. Only once a turbidity was observed, but this I proved² to be non-specific. In spite of the contrary assertion of Magnus and Friedenthal³ I must therefore still adhere to the statement that rabbits do not form precipitins after injection of pollen. These interesting experiments are however being continued. In horses also we do not as a rule observe precipitins. A turbidity was only produced by the serum of one animal⁴ which soon after died of tetanus.

It is interesting⁵, in view of this observation, that the male sexual

¹ Walther, "Über das Vorkommen des Heufiebers beim Pferde," *Berlin. Tierärztl. Wochens.* 1911, p. 818.

² Dunbar, "Über das serobiologische Verhalten der Geschlechtszellen," *Zeitschr. für Immunitätsforschung*, 1910, vol. vii.

³ Magnus and Friedenthal, *Ibid.* 1910, vol. v.

⁴ Dunbar, *Ibid.* 1910, vol. vi.

⁵ Dunbar, *Ibid.* 1910, vol. vi.

cells of fishes and other animals also do not induce the formation of precipitins.

Peculiar conditions are also found with the method of *complement deviation*. Rabbit sera were obtained which gave a complete positive reaction with a 50,000 fold dilution of pollen proteid. Yet in horses, sera of high toxin-neutralising potency only showed a trace of deviation with proteid solutions diluted 1 in 10 or 1 in 100. Sera showing such differences in complement deviation may however possess the same potency in neutralising the corresponding pollen poison for the eye of the hay-fever patient.

The *potency of a serum* cannot therefore be measured by the complement deviation or the precipitin test alone. The former method might incidentally serve to show an increase in the amount of immune substances.

Since all my endeavours to find an animal susceptible to the pollen toxin have failed hitherto, we can at present only ascertain the effect of the pollen-immune serum in the hay-fever patient. In them however the reaction is so definite and precise that this test is one of the most delicate biological methods known to me. Other antitoxins are tested by the criterion of the death or survival of the animal. But in testing pollen antitoxin the hay-fever patient can judge of the existence of the slightest trace of subjective irritation, whilst the investigator watches whether the conjunctiva is congested or remains unaffected. An objectively visible reaction is caused as we have seen by minute quantities of free toxin. In most patients $\frac{1}{200}$ milligramme of the pollen proteid suffices to produce congestion and marked oedema of the conjunctiva. This reaction as will be shown subsequently, usually spreads to the nasal mucous membrane and causes convulsive sneezing fits. In very susceptible patients $\frac{1}{40000}$ milligramme of the proteid suffices to cause the same symptoms. Of the proteid purified according to Kammann's method one drop of a solution 1 in 300 millions ($\frac{1}{50000000}$ milligramme) still caused subjective symptoms in particularly sensitive individuals, the result being controlled by instillation of normal saline which had no effect. These quantities are so minute as to be immeasurable with the most delicate scales, the reactions are therefore far more delicate than those employed in the finest chemical methods. Further, the same patient after many repeated reactions reacts with almost absolute constancy. Privatdocent Dr Carl Prausnitz who for years assisted me in the most self-sacrificing manner in my hay-fever work recently stated¹ that he had carried out thousands of such reactions upon himself without observing any noticeable change in his susceptibility.

Kammann and Gaetgens² have recently found as the result of accurate investigation that a patient who usually reacts to a dilution 1:400,000 may occasionally react only to a drop of a 200,000 fold dilution.

¹ Prausnitz, "Heufiebergift und Heufieberserum." *Habilitationschrift*, Jena, 1912.

² Kammann and Gaetgens. "Experimentelle Untersuchungen über die Bindung von Pollentoxin und Antitoxin." *Zeitschr. f. Immunitätsforschung*, 1912.

In such experiments we have always found the toxic effect to be localised. It never spreads from one eye to the other. But it is washed down from the conjunctiva into the nasal cavity through the nasal duct; thus in all relatively severe conjunctival reactions similar irritation follows in the corresponding side of the nose, but never on the other side. If the toxin is instilled immediately into the nasal cavity, its effect is frequently seen in the posterior pharyngeal part, and some patients even feel irritation in the deeper air passages. Occasionally the toxic effect can even spread in this direction from the conjunctiva down into the pharynx, but it is always confined to the corresponding side.

These observations are the foundation on which I have based the *examination of the antitoxic value of pollen anti-sera*.

The first thing was to determine the patient's susceptibility, *i.e.* the limit of stimulation at which subjective and objective hay-fever symptoms are manifest. If *e.g.* a hay-fever patient did not react objectively to a grass pollen proteid solution 1:30,000, whilst congestion of the conjunctiva was promptly produced by instillation of a drop of 1:20,000 solution, the potency of the serum was determined as follows. Mixtures of the active toxin solution were prepared (*a*) with normal horse serum, (*b*) with the immune serum to be tested. One mixture was instilled into the right, the other into the left conjunctival sac. The mixture containing normal serum was always found to be as active as the pollen toxin alone. But the mixture with immune serum produced no symptoms if the correct proportion was found. Dr Prausnitz, to whom I am indebted for his assistance in these investigations, suggested an improvement of this method which I have regularly employed. The procedure is therefore as follows.

In order to have definite results the toxin solution is used in double the necessary concentration. If the patient shows an objective reaction with a solution 1:40,000, a solution 1:10,000 is prepared. If the serum is supposed to be "40 fold," a dilution 1:20 is made. Equal parts of these solutions being mixed, the toxin is present in 20,000 fold, and the serum in 40 fold dilution. The mixture is kept for 30 minutes at 37° C. and then tested on the patient. If the serum is really 40 fold, objective or subjective symptoms occur. If the patient feels slight itching, but no objective signs are observed, the serum is registered as "bordering on 40 fold." If objective symptoms are seen, the serum is next tested for 30 fold potency, and so on.

The nature of the hay-fever poison and antidote.

For hay-fever patients the proteid of active pollen is a toxin comparable to abrin, ricin, diphtheria toxin, etc. The correctness of this view I have never questioned. But other authors have asserted repeatedly that the pollen proteid is not a genuine toxin. This assertion is based partly upon confused and erroneous views of the facts which had been experimentally proved. My horses were stated after inoculation of pollen toxin to have become not immune, but on the contrary supersensitive. This has already been shown to be a misconception. The only objection which hitherto appeared to be at least partially justified was that according to experiments of Prausnitz¹ the curve of neutralisation of toxin and antitoxin was different from that observed with diphtheria and other genuine toxins. This objection is however not justified since Kammann's² re-examinations showed that with pollen toxin also the curve of neutralisation follows the law of multiples, exactly as with diphtheria toxin. In view of the importance which has been attached by certain authors to this curve of neutralisation, Kammann's technique and results are given in detail.

Results after 3-4 hours' contact of toxin and antitoxin at 37° C.

Minimal active dose: 0.008 mg. = 1 drop of toxin solution 1:5000.

Amount of toxin	Amount of anti-toxin	Result
0.02 mg.	0.2 mg.	neutral
0.4	4.0	trace
0.8	8.0	neutral

The contrary results of Prausnitz are explained by Kammann to be explicable by the use of too small doses of toxin, and by an insufficient time having been afforded to the antitoxin to complete the *chemical* neutralisation of the toxin.

Kammann's experiments were performed with ambrosia-toxin. I consequently requested Drs Gaetgens and Kammann to carry out an extensive series of experiments with grass pollen toxin upon several hay-fever patients. These experiments which were throughout performed

¹ Prausnitz, "Zur Natur d. Heufiebergiftes u. seines spezif. Gegengiftes." *Berl. klin. Wochenschr.* 1905. Prausnitz, "Heufiebergift u. Heufieberserum." *Habilitations-schrift*, 1912, Jena.

² Kammann, "Das Heufieber u. seine Serumbehandlung." *Berl. klin. Wochenschr.* 1906.

under my control and supervision had a decisive result, as will be seen from the following table¹.

To explain this table a few remarks are required. The smallest dose of poison causing subjective and objective symptoms was designated "1 T." Its value varies according to the patient. The details being given in the paper published by these authors. Although the different patients thus required different amounts of toxin, yet the necessary quantities of antitoxin were always the same. This is explained by the consideration that a "40 fold" serum always proves of this strength no matter on what patients it may be tested, even though the minimal toxic dose was very different. Thus in employing the multiples indicated in the table, considerable differences occurred in the amount of toxin which had to be given to different patients. In case I, *e.g.* the 80 fold multiple was $\frac{1}{75}$ mg. toxin, because the minimal toxic dose was $\frac{1}{6000}$ mg. In case IV the same multiple was only $\frac{1}{375}$ mg. because the minimal dose was $\frac{1}{30000}$ mg. This paradoxical observation has proved correct throughout this series of experiments. If in the different patients the dose was increased only slightly above the dose of toxin thus determined, neutralisation was not obtained with these multiples.

"5 T" in the second column means five times the minimal toxic dose, "10 T" ten times the minimal dose, etc.

"1 A" designates the amount of antitoxin required to neutralise 1 T. With the antitoxin employed it was always $\frac{1}{5}$ mg. and with the 80 fold multiple in all cases $66\frac{2}{3}$ mg.

By this explanation the following table is readily understood. It shows that a toxin dose up to 80 T could be completely neutralised by 80 A. Only in some cases did a border reaction occur with the lower multiple, *i.e.* the patients stated that they felt a little itching, without the occurrence of objective symptoms.

*Experiments upon the ratio of neutralisation existing between
pollen toxin and antitoxin.*

Designation of patient	Minimal toxic dose	Multiple tested					
		1 T+1 A	5 T+5 A	10 T+10 A	20 T+20 A	40 T+40 A	80 T+80 A
I Ca	$\frac{1}{6000}$ mg. toxin	0*	0	0	0	0	0
II Chr	$\frac{1}{30000}$ "	0	0 - G†	0	0 - G	0	0
III Kl	$\frac{1}{12000}$ "	0	0	0 - G	0 - G	0	0
IV Ti	$\frac{1}{30000}$ "	0	0	not tested	not tested	0 - G	0
V Vö	$\frac{1}{71000}$ "	0	0 - G	0	0	0	0

* 0 = complete neutralisation.

† 0 - G = boundary reaction (subjective symptoms, but no objective signs).

¹ Kannmann and Gaeltgens, "Experimentelle Untersuchungen über die Bindung von Pollentoxin und Antitoxin." *Zeitschr. f. Immunitätsforschung*, 1912.

The difference between these results and those obtained by Prausnitz is of considerable practical importance, since according to Prausnitz' curve the specific antitoxic treatment of hay-fever patients would hardly appear promising. For it is shown by his curve that—assuming his results to be correct—even relatively small amounts of toxin could hardly be neutralised under ordinary conditions of practice. According to Prausnitz, 2 *T* would require 3·8 *A*, but 5 *T* would need 125 *A*. Yet according to our most recent experiments 2 *T* requires 2 *A* ; 5 *T*—5 *A*, 60 *T*—60 *A*.

This agrees entirely with the satisfactory clinical result obtained with the antitoxin. My views have been further confirmed by experiments performed on man, which I shall discuss later on. Although this question would appear to be definitely decided by the experiments just described, I will mention, for the sake of completeness, a few different hypotheses contained in our recent literature.

The view has been expressed that pollen toxin is an "*endotoxin*," i.e. a substance which is not in itself poisonous but which contains a specific poison. According to this view the hay-fever patient would liberate this toxin by means of a special solvent property of his secretions, and thus develop the symptoms of the disease; on the other hand normal persons would remain unaffected, their tears not having this power of liberating the poison. You are doubtless aware that Friedemann¹ and Friedberger² have been able *in vitro* to liberate *anaphylactic toxin* from substances devoid of any poisonous action, e.g. rabbit's blood or sheep's serum. By employing Friedberger's technique I was able to obtain *in vitro* from pollen proteid with pollen antitoxin and complements, a poison which kills guinea-pigs with the phenomenon of the anaphylactic shock. But this observation cannot be considered as a proof of the endotoxin hypothesis. For this poison did not cause hay-fever symptoms in normal persons. Further, it could not be regarded as a specific poison, because it was also obtained when the specific antitoxin was replaced by normal horse serum. It was even possible to prepare an intense poison for the guinea-pig by merely adding normal guinea-pig's blood to the pollen proteid.

C. Prausnitz³ has propounded a different explanation of the nature of the hay-fever poison and antidote. According to his view, the hay-fever disposition is due to the presence in the hay-fever patient of a very small amount of amboceptor specific for pollen. The pollen proteid coming in contact with the mucous membrane is partially

¹ U. Friedemann, "Weitere Untersuchungen über den Mechanismus der Anaphylaxie." *Zeitschr. f. Immunitätsforschung*, 1909, vol. II. p. 591.

² E. Friedberger, "Weitere Untersuchungen über Eiweissanaphylaxie." *Ibid.* 1910, vol. IV. p. 636.

³ Prausnitz, "Heufiebergift u. Heufieberserum." *Habilitationsschrift*, Jena, 1912.

decomposed by the joint action of such amboceptor and complement; in the further course of events this decomposition would end in the formation of non-toxic substances. Given a sufficient amount of amboceptor, this process would run so rapid a course that the intermediate toxic substance has no chance of coming into action. Thus my specific pollen antitoxin would only influence the hay-fever attack because it adds sufficient amboceptor so that the intermediate poison is prevented from acting.

This hypothesis I do not agree with for the following reasons. The pollen toxin is thermo-resistant, pollen antitoxin is not. If a neutral mixture of the two, which does not affect hay-fever patients, be heated for half an hour to 75° C., the unstable antitoxin is destroyed, but not the toxin. If such a heated mixture is applied to the eye of a hay-fever patient, it causes symptoms of the disease. Thus, the poison is not decomposed by the antitoxin but it only forms a chemical compound with it, from which it was recovered in active form.

Such and similar considerations have firmly convinced me that pollen toxin and antitoxin belong to the class of genuine toxins and antitoxins, like the diphtheria toxin and its antidote. To my mind they act and combine according to the same laws as the diphtheria toxin and its antitoxin.

The present state of our knowledge of hay-fever.

Treatment.

Th. Albrecht declares that ten years ago every physician had his own hay-fever hypothesis. From my own experience I may add that every hay-fever patient also had an hypothesis of his own, which as a rule was most complicated. According to the very detailed information which I have received many patients have employed ten or more hay-fever remedies simultaneously or successively. Every new drug is taken up by the hay-fever patient and enthusiastically recommended to others. For usually he does not hear of it until near the end of the hay-fever season; as soon as he begins to use the remedy, the disease naturally ceases, and he concludes that this is due to the remedy used. Next spring brings the inevitable disappointment. Thus one remedy after another is soon forgotten, but they all reappear, under fresh names. The only lasting drugs are the narcotics, *e.g.* cocaine, adrenalin, anaesthesine, morphine, etc. I need waste no words as to the dangers which follow the repeated use of narcotics. In addition adrenalin and anaesthesine

as well as the numerous preparations embodying these substances produce in many hay-fever patients symptoms even more intolerable than the disease itself.

I have myself so far as possible tried all the remedies which have been repeatedly recommended during the last ten years, but in every case without any result; nor was there any theoretical support for the remedial action asserted. The outcome of my own experience agrees with the result of careful study of the case histories of hay-fever at my disposal. I have mentioned that no success could be expected from any of them and that not even chance has yet provided us with an efficient chemical preparation. Every conscientious physician must warn his patients against the use of narcotics particularly in hay-fever. Therefore the medicines and modes of treatment based on their use need not be entered into.

In over a thousand histories which have been communicated to me by hay-fever patients, the application of caustics, the cautery, chisel and saw in the nose play an important part. Yet all these histories end with the remark that not one of these painful proceedings had benefited the patient. Ten years ago Zarniko declared that such operative treatment having proved useless would cease as soon as a specific treatment had been discovered. This conclusion is as logical and concise as could be desired, yet from a consideration of the histories at my disposal I cannot but presume that even nowadays hay-fever patients are freely operated upon.

It has been shown that the active pollen proteid is a substance of a very high degree of specificity. Thus a proteid causing hay-fever with one patient (*e.g.* grass pollen in Europeans) is inactive in others (patients suffering from American autumnal catarrh). On the other hand, European patients are usually not affected by ragweed proteid. With the complement deviation test I found¹ that this specificity of the different proteids is manifested also in their haemolytic effects, the grass pollen proteids reacting quite differently to those of golden-rod and ragweed. Under these conditions it would indeed be a particularly lucky chance which gave us an efficient remedy: for such a substance would either require to have an affinity to the active proteid—and thus to neutralise or destroy it—or it would have to remove the factors underlying that individual susceptibility which also was shown to be specific.

¹ Dunbar, "Ueber das serobiologische Verhalten der Geschlechtszellen." *Zeitschr. f. Immunitätsf.* 1910, vol. vi.

Since the discovery of the etiology of hay-fever it has been evident that there are only three ways by which the disease can be successfully treated.

The first is to search for localities free from the specific agent; the second to employ apparatus to protect the eyes, nose and mouth of patients from contact with such agents; the third to immunise the patient actively against pollen toxin or to use a specific antidote.

The first way is successfully employed every year by a number of patients. The second also is stated to give satisfactory results.

That hay-fever patients remain free from the disease *if they live in districts where the active pollen does not exist*, is evident from what has been said about the etiology. Thousands of patients find relief and cure of their troubles at sea, on islands or in barren mountain districts. The German Hay-Fever Association recommends Heligoland, in the United States patients retire chiefly to Fire Island, Long Beach Island, the White Mountains, the Green Mountains, the Adirondacks, etc.

Various investigators have recommended masks or *air filters* to be fixed in the nostrils. Verworn recommends the application of a neutral fat (bormelin) to the nasal mucous membrane and the introduction of a cotton wool pledget. He has succeeded in keeping free from attacks by this simple technique. The principle of the treatment is due no doubt to the filtration of the pollen by the cotton wool, for it has been conclusively proved that all non-specific ointments are of no lasting use. On the whole, masks and filters do not appear to have found much favour, the majority of patients being doubtless inconvenienced by such appliances. Neither method of course leads to a permanent cure.

With experiments on active immunisation I shall have to deal later on and will first consider the subject of *passive immunity*.

The efficacy of pollen antitoxin, "Pollantin," having been experimentally decided, there was reason to hope that it would act curatively as well as prophylactically. Soon after the introduction of the specific antitoxin, there followed another serum preparation called "Graminol." In connection with this preparation it was asserted that an efficient serum could be obtained normally from ruminants, *e.g.* cattle, during the time of flowering of the grasses. Kammann¹ and Prausnitz² were able to prove the incorrectness of this assertion and showed that pollen proteid does not penetrate into the circulation from the alimentary

¹ Kammann, "Das Heufieber und seine Serumbehandlung." *Berl. klin. Wochenschr.* 1906.

² Prausnitz, "Heufiebergift u. Heufieberserum." *Habilitationsschrift*, Jena, 1912.

canal, and cannot therefore produce antitoxin. Besides it has repeatedly been found that Graminol has no antitoxic property whatever. A certain number of hay-fever patients have stated that they were successful with Graminol. One author¹ has pointed out that the manufacture of this substance is a trade secret and suggests that it might contain adrenaline. Others interested in the preparation endeavour to explain such results by denying the toxic character of the pollen proteid. These statements can now be considered as disproved and do not require any further discussion.

The specific pollen antitoxin is manufactured under the name of "Pollantin²." It is prepared firstly as (1) pollantinum liquidum: antitoxic horse serum + $\frac{1}{4}\%$ phenol. This preparation easily decomposes and the carbolic odour is unpleasant to many patients. For these reasons the (2) powdered pollantin has been prepared. Antitoxic serum is dried in a vacuum apparatus, powdered, and in this state keeps indefinitely without the addition of any antiseptic. The pure powder is liable to irritate the mucous membrane mechanically but this is prevented by the addition of lactose. Recently a new preparation has been manufactured, (3) pollantin R., specially designed for use in patients who are supersensitive, *i.e.* anaphylactic, to horse serum. At the desire of several physicians pollantin is also made up in the form of (4) an ointment. Lastly I have arranged for the preparation of (5) pollantin pastilles which have been used successfully by several patients against the asthmatic symptoms.

The mode of employment of these substances is very simple. The fluid as well as the powdered pollantin and the ointment is applied in *minute* quantities to the mucous membranes of the eyes, nose and mouth. It is important to do this *before the onset of symptoms of irritation*. This fundamental principle is neglected by many patients.

When the mucous membranes have become congested and oedematous, the antitoxin ceases to be absorbed by them. On the other hand it can only be administered locally, subcutaneous or intravenous application being impossible because the effect of the serum would not last for more than a day or two. The repeated injection of a foreign serum cannot be practised owing to the danger of serum-anaphylaxis. This point I shall refer to presently.

¹ R. Hoffmann, "Beitrag zur Lehre u. Ther. d. Heufiebers." *Mon. f. Ohrenheilk. u. Laryngol.* 1910, vol. v. p. 883.

² Manufactured by Messrs Schimmel & Co. in Miltitz; Agents for the United States, Messrs Fritzsche Bros., New York.

A second mistake commonly made by hay-fever patients is the use of far *too large quantities* of pollantin. From the very first I laid great stress upon the application of only minute amounts of the substance. The antitoxic effect of pollantin is so considerable, that a particle of the powder suffices to neutralise all the pollen toxin which can attack a patient in the course of a day. The employment of unnecessarily large doses renders many patients supersensitive (anaphylactic) to horse serum.

Such symptoms of horse serum anaphylaxis were observed by some patients as early as 1905. At that time our knowledge of this condition was comparatively slight. Even at that time I¹ was able to prove that the irritative effect of the serum had no connection with its antitoxic potency, for these patients showed the same reaction when *normal* horse serum was applied to their mucous membranes. It was noteworthy that hay-fever patients could render their whole body anaphylactic by simple treatment of the conjunctival membrane, the condition being shown by the uninjured skin reacting with itching, burning and erythema to the instillation of a drop of normal horse serum, which thus acts practically like the pollen proteid. Similarly in hay-fever patients who have become anaphylactic, horse serum affects the conjunctival and nasal membranes exactly like pollen toxin. That is the reason why several patients wrote to me that antitoxin caused or increased the hay-fever attack instead of curing it. In every case where normal serum was tested patients admitted that it also produced symptoms resembling hay-fever.

As a matter of fact horse serum anaphylaxis does not seem to occur very commonly among hay-fever patients—which individuals I used to regard as specially liable to this affection—the first impression I received from a large amount of direct correspondence was that it must be a fairly prevalent condition. But an enquiry instituted recently has shown that a very large percentage has used the remedy every year without being in the least irritated by it. Nor would thousands of hay-fever patients employ it every year if they had to suffer from anaphylactic symptoms; the majority of them are far too impatient for this supposition to be probable. For many years I have endeavoured to remove these irritating substances from the antitoxin—hitherto in vain. There was not indeed much hope of success, for as early as 1905 I found this property was connected with the euglobulin of the serum

¹ Dunbar, "Aetiologie u. spez. Ther. d. Heufiebers." *Berl. klin. Woch.* 1905, Nos. 26, 28-30.

as is the antitoxin itself. If therefore you destroy the englobulin—from which the anaphylaxia-producing bodies are inseparable—you also break down the antitoxin.

There are two ways by which I was able to help patients who had thus acquired anaphylaxis. One was the employment of the diluted preparation (pollantin R.) together with the advice to use this preparation only *before* the beginning of an attack, and to apply it in most minute quantities once a day or if possible at still longer intervals. Patients who tried this procedure informed me that the preparation did not irritate them at first but did do so later on. This agrees with my own experience. They found the irritation however to be tolerable and to disappear after 20 or 30 minutes, the result being that they afterwards remained free from attacks for the whole or even several days.

Another method was based upon the fact that horse serum anaphylaxis is specific in most, though not in all persons. Thus I have heard of cases where anaphylaxis having developed against one animal serum, patients also became supersensitive to others. But that is an exceptional condition. For this reason I supplied patients irritated by pollantin R. with a highly potent rabbit serum, the results being excellent. They were not only quite unaffected by the serum but also remained hay-fever free in the midst of the season. Unfortunately however in such patients supersensitiveness to rabbit serum also developed after a time.

Several years ago I suggested that in the same measure in which sensitiveness to sera is increased, the reaction to antitoxin would also grow, together with a tendency towards definite immunisation. Persons who have become anaphylactic in my experience require far smaller quantities of antitoxin than other patients, and have, I believe, better chances of definitely losing their susceptibility.

This of course must be the aim of specific treatment in hay-fever. Years ago I learnt of several patients, some of them very severe cases, who after comparatively short use of pollantin ceased to suffer from hay-fever attacks although they used no preventive measures or remedies. Such persons I believe to have been completely cured and I published this view last year¹. In consequence of this publication a nasal specialist interested in hay-fever wrote that he could not understand such results for neither he nor any of his friends had ever observed such cases. In addition, my favourable results were in direct opposition to those of the German Hay-Fever Association. My endeavour to

¹ Dunbar, "Zur Ursache u. spezifische Heilung d. Heufiebers." *Deutsche med. Wochenschr.* 1911, No. 13.

defend myself against such attacks led me to place my material at the disposal of Dr Albrecht, the Secretary of the German Hay-Fever Association. I was agreeably surprised to learn that he himself had seen numerous cases in which pollantin had not merely cured the attacks but had produced real immunisation. In a recent publication¹ he was able to report upon no less than 18 cases in which after a comparatively brief use of the remedy the hay-fever attacks ceased entirely or were at least markedly reduced. These observations appear to me to be of paramount interest in relation to hay-fever and I should be grateful if any colleague who has seen similar cases would communicate them to me.

The occurrence of horse serum anaphylaxis in hay-fever patients using pollantin has led me to pursue my investigations on this subject since 1905. All endeavours to prevent it have been in vain. On the other hand I found that it only occurred in a comparatively small percentage of cases, and that it then is regularly an indication of a decline in the hay-fever predisposition. Several such patients have repeatedly informed me that in spite of using only very small amounts of diluted pollantin they were able to keep themselves in a satisfactory condition throughout the season; they were able to pursue their avocations and often only required to employ the antitoxin at intervals of several days. Nevertheless this condition was not desirable, especially since the alternative use of antitoxin from the rabbit was also soon followed by anaphylaxis. Such experiences convinced me of the desirability of recommencing experiments on *active immunisation*. The chief consideration which caused me to hesitate was connected with the grave symptoms that occurred at the beginning of my experiments, *i.e.* before the time of exact dosage. Subsequently I was doubtful for a time whether hay-fever was an anaphylactic condition, as has been most definitely asserted by several authors. Only recently have these doubts disappeared owing to the toxin neutralisation experiments described previously. I did not expect too much from active immunisation, since every hay-fever patient is regularly exposed to the action of pollen toxin for six or eight weeks every year; thus one would expect him to be actively immunised by natural means were this at all possible. A friend has carried out systematic experiments to reduce the conjunctival sensitiveness by systematically instilling into the conjunctival membrane increasing amounts of toxin: these experiments were unsuccessful.

¹ Albrecht, "Immunisierung gegen Heufieber." *Deutsche med Wochenschr.* 1912.

I was thus led to suppose that a result might possibly be achieved by employing neutralised mixtures of toxin and antitoxin. The result of passive immunisation, *i.e.* by employing antitoxin, seemed to favour this view. For the pollen toxin to which these patients were normally exposed year by year did not cure them; but definite immunity occurred as soon as the toxin was properly neutralised by the correct use of antitoxin throughout the hay-fever season. Before beginning to immunise patients with neutral mixtures of pollen toxin and antitoxin I hesitated for a time from fear of horse serum anaphylaxis. But experienced physicians, particularly specialists in children's diseases, reassured me by the information that they had for years given many of their patients subcutaneous injections of very large quantities of diphtheria antitoxic serum from the horse without witnessing anaphylactic symptoms. I therefore began these experiments by giving a neutralised mixture of grass pollen toxin and antitoxin subcutaneously to a patient who had for a considerable time used pollantin successfully without becoming supersensitive to horse serum. The injection was borne well. At the site of injection a slight sensation of warmth was felt, but no itching and burning and no other morbid symptoms followed. The only noticeable feature was a swelling about two inches in diameter round the injection which disappeared after 24 hours. Before treatment the patient showed a subjective and objective reaction with one drop of pollen toxin "Ka. VII" diluted 1:10,000,000. He received altogether 15 injections lasting well into the hay-fever period. The local reactions were less after the second injection and remained slight with the later ones, although the amount of toxin was doubled each time. The first injection was performed with a neutralised solution of toxin 1:50,000,000, the last with a solution of 1:40,000.

The immunising effect of such treatment was proved by the fact that in spite of this rapid increase in the dose no hay-fever symptoms followed the injection and the local reaction diminished. The limit of toxic action on the conjunctiva was reduced from 1:10,000,000 to 1:100,000.

The patient had been known to me for a long time; his relatives had frequently complained how intolerably he suffered during the hay-fever season. In this year no symptoms occurred at the time when other hay-fever patients were suffering and the number of grass pollen in the air was found by Dr Gahtgens to have reached a considerable level. At my suggestion the patient took long walks and even went to Thuringia for a six days' trip in the early part of June, *i.e.* during the

bad season for hay-fever patients, and was in the open all the time. Yet he remained practically free until the return journey. In the train he suffered from several rather severe attacks which rapidly ceased after the use of pollantin. The symptoms were practically limited to sneezing, he had felt remarkably free from any sensation of illness which formerly used to trouble him greatly. Immediately after his return to Hamburg he resumed his long walks in the open and only on seven days suffered from somewhat severe sneezing attacks which he was almost always able to relieve promptly by pollantin.

I propose to treat several other patients with neutralised toxin antitoxin mixtures in order to determine whether permanent immunity can be obtained by this treatment corresponding to that described in Dr Albrecht's cases. I am particularly interested in comparing such results with those obtained by purely active immunisation.

However successful this method may have been, it was not applicable in those patients who had developed anaphylaxis and who were therefore of particular importance. Those patients who are completely successful with the simple external application of pollen antitoxin and can keep free from attacks by this means alone have no reason to exchange it for a more complicated and more expensive method of treatment.

The active immunisation of hay-fever patients appeared somewhat hopeless at the beginning of my experiments since hay-fever patients do not become immunised during their yearly period of illness. The view that the body would not react to the local attack of pollen which normally settles on the mucous membranes, but would respond with the production of anti-bodies to the direct injection of the poison into the subcutaneous tissues, appeared improbable, since I had proved that pollen toxin occasionally penetrates through the skin or mucous membrane and enters the body in the form of a genuine proteid. Since then I have found such penetration to occur but rarely. The results of local active immunisation of the conjunctiva mentioned previously were also unsuccessful. Yet Robert Koch, who took a considerable interest in my hay-fever investigations, repeatedly advised me to recommence experiments with active immunisation, beginning with minute doses.

Recently such experiments have been undertaken in Sir Almroth Wright's laboratory by L. Noon¹ and J. Freeman². Several hay-fever

¹ L. Noon, "Prophylactic inoculation against hay-fever." *Lancet*, 1911, vol. 1, p. 1572.

² J. Freeman, *Ibid.* vol. II, p. 814.

patients were injected subcutaneously with increasing quantities of pollen extract at intervals of three to 14 days. The susceptibility of the conjunctiva was found to decline during this treatment. If too large doses of the poison were given, the susceptibility showed a passing increase (negative phase of Wright). The authors were able to reduce the susceptibility to about $\frac{1}{100}$ of the original, *i.e.* to a limit which doubtless would suffice to render many patients resistant to the effect of the pollen quantities to which they are normally exposed.

The experiments of Noon were continued by Freeman on 20 patients. He also reduced the susceptibility of the conjunctiva to between $\frac{1}{10}$ and $\frac{1}{100}$ of the original. Three patients seemed to have had a satisfactory result, 13 were markedly improved, whilst two cases were not influenced. The reduction of conjunctival susceptibility bore a certain analogy to the clinical effect.

This result has been regarded by others as an anti-anaphylactic condition. I also was at first inclined to fear that an artificial immunity obtained by a purely active method would have no lasting effect. Freeman has since informed me that his results were satisfactory in 1912. In persons, who had been actively immunised in the previous year, the degree of immunity which had fallen considerably during the winter was raised to a very high level without any unpleasant symptoms by means of a single injection.

My experiments were recommenced in April 1912, such patients being chiefly selected who had become highly anaphylactic to horse serum. I also treated the severest cases that I knew, patients who complained that they had to give up their profession owing to hay-fever, that throughout the season they found no peace at night from asthmatic symptoms and in the day time were unable to leave the room.

I have altogether treated ten patients, one of these being the case already mentioned where toxin antitoxin mixtures were administered. At the same time a person not affected by hay-fever was also injected subcutaneously with increasing doses of pollen toxin. This individual, as was to be expected, did not acquire hay-fever, and the injections were followed by no symptoms whatever either before or during the hay-fever period.

The treatment of most of the patients was begun between the 29th April and 8th May, *i.e.* several weeks before the beginning of the hay-fever period. This was ascertained for Hamburg to be the 30th May. In two patients treatment did not begin till the 7th and

11th June respectively, *i.e.* at the worst time of our hay-fever period when the rye was in full flower.

The first dose in all patients was chosen according to their susceptibility as determined by the ophthalmo reaction; if *e.g.* the conjunctiva reacted to one drop of a toxin solution 1:100,000,000, the patient was injected with 1 c.c. of a solution 1:300,000,000, and so on. All patients reacted to the first injection with a swelling at the site of injection, which was one to six inches in length, hard, red, hot, and itching. The toxin doses were doubled every time, yet at the second injection performed five or six days later, the local reaction was already less marked in several of the patients, and at the subsequent injections it was either quite absent or at any rate slight.

Only in my own case and in that of another very highly susceptible patient who used to suffer from severe asthma, a rapid increase in the dose of toxin was followed by general symptoms in addition to the local reaction. These general symptoms correspond with those described in an earlier publication¹, so that I need not enter into details in regard to them. They however always remained within moderate limits and were less distressing than a typical hay-fever attack, excepting perhaps an oedematous swelling of the eyelids which lasted for two days.

In my case the toxin dose was increased to 1500 times the original, the reactions being considerably less than after the first dose. In the other patients the concluding dose was increased to between 50 and 2000 times the original dose. These high multiples either produced no reaction or at least less intense symptoms than the initial dose.

During the hay-fever season the following observations were made on the seven patients who had begun treatment before the time of their illness.

I have myself for a number of years possessed a certain degree of immunity; thus I only suffered from slight transitory attacks of sneezing when I was much in the open or travelled by train on days when the pollen count in the air was high. In this year I was able to remain out of doors even on such days without experiencing any attacks. A journey of three weeks duration, in the course of which I was almost daily moving about among flowering meadows, conclusively showed that through the systematic toxin injections my susceptibility had been still further diminished.

Of the remaining patients only one had shown an indication of immunity in the year before. All the other cases were severe and

¹ Dunbar, *Zur Ursache u. spez. Heilg. d. Heuf.* München, 1903, pp. 32-35.

complicated either by horse serum anaphylaxis or by pollen asthma. They all declared that formerly they had suffered daily for six or eight weeks, even if they did not leave the house and in spite of an exhaustive application of all manner of pharmaceutical remedies. Even in this small number of cases I have seen how difficult it is to obtain exact numerical replies as to the effect of treatment in hay-fever.

Thus one patient stated early in June that he could remember no year in which he had been so free from attacks as this one, in spite of undertaking things he would not have done previously, *e.g.* railway journeys etc. At the end of treatment, however, he asserted that the whole treatment had not benefited him in the least. Yet when reminded of his original statement, he admitted that the attacks had been less frequent and less severe than in previous years.

A second case was quite similar. Although the patient did not say that the treatment had not benefited him at all, he still did not think very much of it.

In a third patient the treatment was interrupted on the 10th June because he had to leave town. Up to then he had been out of doors a great deal during the worst part of the hay-fever season as a yachtsman, but had suffered only from very mild sneezing attacks. He was himself convinced that he had had far better results than ever before.

Another patient used to suffer—in spite of all precautions—from a most intense feeling of illness in addition to the ordinary symptoms of sneezing, watering of the eyes, etc. Although he took fewer precautions this year and was very much out of doors, he suffered only on six days with mild sneezing attacks and only on one day experienced a characteristic feeling of illness. Another case was quite similar. A third patient had formerly been forced to remain in doors throughout the season, was unable to follow his profession, and yet suffered severely from asthma. In this season he was able to follow his profession all the time. What is more, he travelled about by motor and by train, yet only had mild or moderate sneezing attacks on five days, *i.e.* three or four sneezes without any sensation of illness. Such sensation of illness was experienced only on one day in this year. It was associated with moderate asthma and followed a two hours motor drive on the 29th June. Now on this day our pollen counts in the centre of the city had given the amount of 273,000 pollen per square metre. In all these patients the susceptibility had fallen to between $\frac{1}{20}$ and $\frac{1}{100}$ of the original.

Of the two cases where treatment was begun in the middle of the

hay-fever season, one experienced no benefit. The other patient remained entirely free from attacks after the 30th June, *i.e.* about three weeks after the beginning of the treatment, although he lives in a house surrounded by meadows and is forced by his profession to be out of doors a great deal.

The foregoing remarks show that experiments with active immunisation in hay-fever appear hopeful. Statistics will doubtless be far more satisfactory, as soon as the experiments are extended to ordinary cases, not being confined to the most severe ones. Further it should not be lost sight of that in the latter we were unable to commence treatment until four weeks before the beginning of the disease. A further considerable improvement in our results may be expected, if inoculation is begun several months before the hay-fever season, as was done by Noon and Freeman.

Both from practical and scientific considerations it was important to prove that the injections can be performed without any annoyance worth mentioning being caused to the patient. Still I would strongly advise that they should never be undertaken before the limits of the patient's susceptibility by the ophthalmo-reaction has been ascertained.

The results obtained appear so satisfactory to me that I propose extending my investigations on a considerably larger scale in the future.

THE PATHOLOGICAL CHANGES IN PELLAGRA AND THE PRODUCTION OF THE DISEASE IN LOWER ANIMALS.

BY LUCIUS NICHOLLS, M.B.

(From the Quick Laboratory, University of Cambridge.)

(With Plates IV and V.)

ALMOST from the time when pellagra was first described, a connection between the disease and maize as an article of diet has been noted, and a wordy and voluminous literature has arisen in the countries afflicted concerning the nature of this suggested relationship between maize and pellagra. Most of what has hitherto been written about pellagra is based upon observations on clinical material and epidemics. Probably the most neglected side of the disease is its morbid anatomy and histology; and the effects of feeding animals on preparations of maize are but scantily described.

My description¹ of the pathology of this disease is based on notes of eight post-mortems and microscopical sections of specimens taken from these subjects, and also on observation on an identical condition produced in rats by feeding them on the products of decomposing corn-meal.

It is evidently necessary that the pathological changes in the human being should be well ascertained before an attempt is made to compare them with the changes induced in animals.

In all these cases which I am considering the clinical picture was complete, but though dementia was present in every one of them, in the majority there was little indication of degeneration of the peripheral nerves.

Firstly the condition of these patients during life may be briefly

¹ The experiments described in this paper were carried out in St Lucia, B.W.I. The preparation of the microscopical sections etc. was done in the Quick Laboratory, Cambridge, by the kind permission of Prof. Nuttall, F.R.S.

summarised. All were mulattoes belonging to the lower classes, and though none were white skinned several were relatively light coloured. Five of them were women and three were men, and their ages ranged from twenty-six to seventy-two. From the time when they first came under observation until death their temperatures were usually normal, and a rise up to 100° Fah. occurred only for a short time in one or two cases.

A superficial eruption on the dorsal surfaces of both hands had been present in all cases, and in several there were eruptions on other exposed parts of the body such as the ankles, shins, forearms, face and the back of the neck. A progressive and extreme emaciation was exhibited by all of them. Several had tremors and loss of knee-jerks. The curious, morose and irritable dementia which is almost pathognomonic of this disease was present in all.

They complained of soreness of the mouth and this was due to irregular exfoliation of the epithelial layer of the mucous membrane of the tongue and buccal cavity, which in two cases extended to ulceration at the angles of the lips. Loss of appetite and persistent diarrhoea usually extended through the course of the disease. In the later stages of these cases shrinkage of the liver and spleen could be easily demonstrated.

Traces of albumen and blood corpuscles in the urine occurred in one or two cases.

Two cases had eczematous ulceration between the thighs.

Post-mortem examinations.

The changes found in the bodies after death were of a very definite and uniform nature.

External appearances. All the bodies were extremely emaciated, three of them, notably two women aged thirty-two and thirty-four and a man aged twenty-six, had reached a stage of emaciation not often seen even in the post-mortem room. (These patients had been well cared for in a government institution for at least six weeks.) In each case soreness of the mouth and lips, and an eruption on the exposed surfaces were noted. Two cases, a woman and a man, had exfoliation of the skin between the nates, and this extended down the inner surfaces of the thighs.

Internal examination. On cutting through the skin of the thorax and reflecting it, an almost complete absence of adipose tissue was found, and extreme wasting of the muscles was noted.

Pleurae. In four examinations the pleurae were normal. In three there were a few old adhesions between the visceral and parietal layers. One case showed a few petechial haemorrhages on the pleural surfaces.

Lungs. In two autopsies the condition was normal. In two others there was oedema of the bases. One case showed marked hypostatic congestion, and one patient had died of a terminal pneumonia. Tuberculous areas were present in two cases, but in neither had the condition advanced so far as to produce caseous foci. The microscopical examination showed no changes which could be associated with the disease.

Heart and pericardium. The latter in several cases was of a dull appearance and slightly thickened; the thickening was confirmed by microscopical examination.

In most cases the heart was atrophied as evidenced by the loss in weight (*v. chart*). In one patient the heart appeared enlarged and was dilated, the muscle having undergone fatty degeneration.

Sections demonstrated that there was an increase of fibrous tissue in these hearts and a varying degree of thickening of the walls of the arterioles. In all cases the muscle fibre exhibited brown atrophy.

The aorta and the large vessels. In the three cases in which the patients were under thirty-five years of age, no pathological changes were noticed in the large vessels, the intima being entirely free from any signs of disease. In the two patients aged forty-three and forty-four respectively, there were a few areas in the aorta just above the semilunar valves which showed early degenerative changes; but the carotids and axillary arteries were free from disease.

In the three older patients more or less extensive arterio-sclerotic and atheromatous changes had taken place in the larger vessels.

The peritoneal cavity. The fat in the omentum and mesenteries was reduced to a minimum; the omentum being as thin and friable as tissue paper.

The liver. This organ was much reduced in weight; the average in seven cases in which the scales were used was $37\frac{1}{2}$ ozs.; a very low weight, considering that the average for an African is 52 ozs. and for a European a little more. One of these patients was a heavy mulatto who, previous to this illness, had scaled 160 lbs.; his liver weighed 35 ozs. In all the cases the section of the organ was of a dull mottled colour, but showed neither the nutmeg phenomenon of venous engorgement nor the iodine staining of amyloid disease. Very advanced fatty degeneration had taken place in two patients. In all cases, however, the tissue of the organ showed increased resistance to the knife. There were petechial

haemorrhages under the capsule in some cases, and certain dark spots in the substance which were probably due to small haemorrhages.

Microscopical examination showed fatty degeneration, but only in two cases did this reach an extreme condition (Fig. 4).

In the older and more chronic cases there was an increase in the fibrous tissue around the vessels, and this was accompanied by great thickening of the vascular walls. In places there were areas of venous engorgement. There were general atrophy and degeneration of the liver cells, and often a considerable amount of pigment was present, possibly derived from previous small haemorrhages, for these were occasionally seen among the liver cells.

The spleen. The shrinkage of this organ was phenomenal, especially when it is remembered that all these patients belonged to malarial localities and must have suffered from attacks of malaria. In seven cases in which the organ was weighed, the highest was $5\frac{1}{2}$ ozs. and the lowest was 7 drachms; the average weight was just under 3 ozs.!

The section of the spleen was very dark in colour, and in most cases the fibrous structure appeared to be increased. The tissue was resistant and tough.

The whole condition and appearance of the organ was the very opposite to what occurs in febrile infectious diseases.

The microscopical examination showed that the fibrous tissue of the capsule and of the splenic pulp was increased, and that the arterioles were thickened.

In some cases numerous haemorrhages from the finer vessels produced a very extraordinary condition, since in every field of the microscope the number of free red blood corpuscles far exceeded the number of splenic cells. Moreover, numerous small masses of pigment bore witness to the fact that these haemorrhages had occurred frequently.

The kidneys. Very little change in the kidneys was noticeable, but the organs were decreased in weight. In fact, compared with the obviously advanced degeneration of the liver and spleen, the comparatively natural condition of these organs was striking. In the two oldest patients early granular changes were present.

The suprarenals. In several cases these were very friable but were not shrunk. They did not appear to have taken part in the general wasting of the organs of the body.

The stomach. In all cases the walls of the stomach were thinner than usual. In two cases the organ was considerably dilated. In one case the contents were darkened by altered blood which had come from

two or three minute superficial ulcerations. In several cases minute petechial haemorrhages were present in the mucous membrane.

Normally the muscular coats of the stomach occupy a little more than two-fifths of the thickness of the wall of the viscus, in pellagra they are reduced to about one-third. There were a few areas of capillary stenosis in the mucous and submucous coats and occasional places where diapedesis of red blood cells had taken place.

The small intestine. The wall of the bowel was atrophied and the valvulae conniventes were thin and shrunken, in some cases having almost disappeared. In places the bowel wall was very friable. There were petechial haemorrhages of areas throughout the small intestine, and these were more pronounced the nearer one approached the ileo-caecal valve; in some places where they were in the mucous membrane, the condition somewhat resembled a surface which had been sprinkled with cayenne pepper. These haemorrhages also occurred in the serous covering of the bowel and a few even between the muscular coats. The mucous membrane was, in places, very superficially eroded and had a roughened appearance. The small haemorrhages and the erosion were most marked on the valvulae conniventes.

The large intestine. The condition was similar to that seen in the small intestine, but more exaggerated. In one or two places the mucous membrane was deep purple in colour, owing to the enormous number of capillaries, and small haemorrhages from them.

A single deep ulcer in the rectum was present in two cases.

The microscopical changes in the intestinal tract were of the same nature throughout, but increased in intensity from above downwards.

The atrophy of the muscular coats was such that they occupied about half of the thickness of the bowel wall, whereas normally they occupy about five-sevenths of the thickness of the jejunum and ileum. The mucous membrane was also wasted, and accentuated the muscle atrophy. The villous processes of this coat were very greatly degenerated, and where the degeneration was most advanced, the cells were almost replaced by thin fibrous tissue. Capillary stenosis was present in the deeper layers but was not well marked. In places capillary haemorrhages had occurred. Occasional areas were seen in which erosion of the mucous membrane has led to round cell infiltration and other signs of inflammatory reaction (Fig. 7).

The changes in the submucous coat consisted in an increase in fibrous tissue, degeneration and thickening in the smaller vessels, and

areas where haemorrhages have taken place; the latter occasionally caused local thickening of this coat.

The serous coat was thickened, and a few haemorrhages were present between it and the muscular coat.

The bladder. In two cases the mucous membrane of the bladder was dotted with petechial haemorrhages.

The brain and nervous system. In some cases the dura mater was thickened, and the pia mater showed irregular areas due to thickening. The convolutions of the brain were atrophied, and were of firmer consistence than normal.

In the two oldest patients the arteries at the base of the brain were sclerotic and had calcareous deposits.

Although mental symptoms are always present and many cases exhibit signs of nerve degeneration, it is only in a limited number of cases that a microscopical examination shows much pathological change. In these patients there was an increase in the fibrous tissue in the meninges and the arterioles supplying them were thickened, also the endothelium of the capillary vessels showed degeneration with a tendency to proliferation. The cortical cells of the brain were shrunken and degenerate.

Mott, Batten, Tuzek and others have examined the nervous system in cases of pellagra, and their results show that the cellular degeneration and subsequent sclerotic changes which take place in the other organs are also present in the nervous system in a number of cases. For example an increase in the neuroglial elements of the brain and a sclerosis of the posterior and lateral columns of the spinal cord have been described.

Microscopical examination of the skin at the site of the eruption. There was atrophy of the stratum corneum, with great tendency to desquamation in the exposed parts of the body; by the separation of the stratum corneum from the stratum granulosum blebs may be formed.

In the outer layers of the stratum mucosum irregular cell proliferation and some degeneration occur.

The vessels of the dermis became sclerotic, and there is capillary degeneration and stenosis; occasionally minute haemorrhages occur.

Summary of post-mortem and microscopical findings.

The accompanying table gives a summary of the post-mortem findings in these cases; in the last column the average weights of the organs in these cases are compared with the average weights of the organs of normal individuals.

Patient's initials...	Average weights in these cases.								(usually less than Europeans).
	Case 1	2	3	4	5	6	7	8	
Age ...	M. P. 34	C. C. 60	A. St. C. 58	M. Al. 26	G. A. 43	A. C. 70	T. A. 32	J. M. 44	
Sex ...	Female (Acute)	Female (Chronic)	Female (Acute)	Male (Chronic)	Male (Chronic)	Female (Chronic)	Female (Acute)	Male (Acute)	
External condition	Hands, arms, legs and face	Hands & feet	Hands, face and feet	Hands, legs and feet	Hands, feet, legs, face and neck	Hands and feet	All exposed surfaces	Hands and feet	
Eruption ...	Extreme	Extreme	Great	Extreme	Considerable	Extreme	Extreme	Some	
Emaciation ...	Ulcerated	Ulcerated	Ulcerated	Not as much as usual	Not as much as usual	Ulcerated	Ulcerated	Ulcerated	
Mouth and tongue	Ulcerated	Nil	Nil	Ulcerated	Nil	Nil	Nil	Nil	
Angles of mouth...	Nil	Nil	Present	Present	Nil	Nil	Nil	Nil	
Eczematous condition between thighs	Nil	Nil	Present	Present	Nil	Nil	Nil	Nil	
Internal condition	Nil	Old tuber. lesions	Oedema at bases	Hypostatic congestion	Oedema at bases	Nil	Near two tub. areas	Terminal pneum.	9½ ozs. 13½ ozs. each
Lungs ...	7 & 8 ozs.	6½ & 7 ozs.	12 & 13½ ozs.	11½ & 12 ozs.	10½ & 11 ozs.	5½ & 6½ ozs.	6 & 7 ozs.	16 & 9 ozs.	
Pleurae ...	A few ad-hesions	Old ad-hesions	Nil	Petechial haem.	Old ad-hesions	Nil	Nil	Same fluid	
Pericardium	Myocardial disease	7½ ozs.	9 ozs.	6½ ozs.	6 ozs.	5½ ozs.	7½ ozs. 9 ozs.
Heart ...	7 ozs.	5½ ozs.	13 ozs.	7½ ozs.	9 ozs.	6½ ozs.	6 ozs.	5½ ozs.	
Aorta and large vessels	Natural	Sclerotic	Sclerotic	Natural	A few early changes	Very sclerotic	Natural	A few early changes	
Peritoneal cavity...	Extreme loss of fat in omentum and peritoneum generally.	Shrunken	43 ozs.	35 ozs.	46 ozs.	31 ozs.	35 ozs.	41 ozs.	37½ ozs. 52 ozs.
Liver ...	Shrunken and adv. fatty deg.	Shrunken and fibrous	31 ozs.	1½ ozs.	5 ozs.	1 oz.	2 ozs.	5½ ozs.	3 ozs. 12 ozs.
Spleen ...	Shrunken & very dark	3 & 3½ ozs.	3½ & 3½ ozs.	2½ & 3½ ozs.	4 & 4½ ozs.	2½ & 3 ozs.	2½ & 4 ozs.	3½ ozs.	4½ ozs.
Kidneys	3½ & 3 ozs.	Nil	Nil	Friable	Nil	Nil	Nil	Nil	
Suprarenals	Friable	Nil	Nil	Friable	Nil	Atrophic	Nil	Nil	
Stomach ...	A few pe-tech. haem. walls	Very thin	A few petechial	Very thin	Nil	Atrophic	Nil	Nil	
Small intestine ...	The picture of atrophy, petechial haemorrhages, and patches of surface erosion, was the same in all cases.	
Large intestine ...	Nil	Nil	Petechial	Nil	Nil	Nil	Petechial	Nil	
Bladder	Nil	Nil	Petechial	Nil	Nil	Nil	Petechial	Nil	
Meninges ...	Some thickening and in older cases, atheromatous arteries.	
Brain ...	Firmer than natural, and apparently shrunken in all cases.	

The pathological changes which take place in pellagra are to a great extent due to degeneration of the capillaries and smaller vessels; this gives rise to stenosis and blood stasis which cause leakage or actual haemorrhages from the weakened vessels. This explains the atrophy of the tissues by impaired nutrition, and the eruption on the exposed skin surfaces where solar radiations and possibly other factors accentuate the damage which has taken place in the vascular supply with consequent exfoliation of the stratum corneum, bleb formation from exuding serum, and pigmentation from altered haemoglobin. The tissue cells, especially those of the liver, spleen and alimentary tract are also attacked.

Assuming that the cause of pellagra is a toxin developed by micro-organisms in preparations of maize or possibly other farinaceous material, the pathological changes may be divided into three stages:

(1) The initial stage in which the toxin causes degenerative changes in the endothelium of the smaller vessels, and in the endothelial and glandular cells of the alimentary system.

(2) The acute stage in which the toxin acts in a more widespread manner, causing great damage to the capillaries and arterioles, resulting in repeated haemorrhages; it also acts upon the cells of the organs. It is in this stage that the mental symptoms and the skin eruption first appear.

(3) If the patient survives the acute stage the chronic stage sets in with great fibrosis of the arterioles and capillaries, general sclerotic changes, and much atrophy and degeneration of the cells of the spleen, the liver and the alimentary tract. The toxin may not be present in this stage of the disease, which is a natural result of the damage which has taken place in the acute stage. The eruption may disappear, but reappears on exposure to the sun. The general sclerosis of this period of the disease extends to the spinal cord and nerves, and thus gives rise to the nervous symptoms.

Three hypotheses on the etiology of pellagra at the present time attract some attention:

(1) The maize hypothesis which affirms that the disease is due to deleterious substances present in or formed in corn-meal and its products by micro-organisms.

(2) The sand-fly protozoal hypothesis which postulates that the disease is due to a protozoal organism which is carried from the infected to the healthy by the agency of the sand-fly.

(3) The third hypothesis suggests that the cause of the disease is a bacterium.

The first of these has been ably supported by Lombroso and a host of observers.

The second has more recently been brought forward by Sambon and Chalmers, who (*British Medical Journal*, Oct. 26th, 1912) actually make the following statement: "We have shown that pellagra is an infectious insect-borne disease...." Their evidence in support of the theory is of a very frail nature.

The third hypothesis has been urged by Tizzini, who, at the Italian Pellagra Conference of 1912, stated that in fifty consecutive cases he had isolated a bacillus from the blood. As in other diseases which affect the intestinal tract, blood cultures frequently give positive results due to leakage from the gut.

During pyrexial periods I have been able to cultivate from the blood organisms of the family Typhaceae.

Many attempts have been made to produce pellagra in animals, usually with little success.

Some time ago Raubitshek injected and fed guinea-pigs with extracts of good and damaged maize, but his results were negative; he also worked on fagopyrismus in animals, a disease produced by feeding animals on buckwheat, but the condition is far from analogous to pellagra. In further experiments he believes he demonstrated the presence of photo-dynamic substances in maize, which are allied to those extracted from buckwheat and which produce symptoms of disease.

He appears to lay too much stress upon the skin eruption, and to consider it the principal phenomenon of pellagra; but a toxin which produces a characteristic condition in one animal may be poisonous to another without its most marked symptom appearing. Thus in ergot poisoning the typical gangrene is absent when experimenting with such animals as rats, yet they may die of the poison.

Lombroso experimented with spoiled corn and extracts of it and numerous bacteria obtained from corn. Injections of *Bacterium maidis* (of the potato bacillus group) into white mice produced paralysis, coma and death. Cultures of this organism on polenta, the Italian maize bread, when given to mice produced diarrhoea and other symptoms referable to the alimentary system.

Lombroso and other investigators have produced symptoms resembling pellagra in chickens fed on spoiled corn. It is a well-known fact in certain tropical regions that feeding fowls on mouldy corn frequently causes their death. (I once made a post-mortem on a fowl

which had died after being fed on damaged maize, and the liver, spleen and intestines exhibited many lesions analogous to those seen in the bodies of human beings who have died in the acute stage.)

Hauseman, Erba, Tirelli, Pelizzi, Gosio, Fenati and other Italians have produced disease in fowls and other animals injected with maize extract, or mouldy corn-meal.

Lavinder fed animals on normal corn-meal for several months without producing symptoms of disease.

Anderson and Goldberger have attempted with practically no result to produce pellagra in monkeys by the injection of the blood or spinal fluid of patients suffering from the disease.

Babes and Manicadide describe experiments which they believe demonstrate that the blood of pellagrins can neutralise the toxins found in spoiled maize.

To expect that the exact picture or all the signs and symptoms which occur in man will be obtained in these experiments is to expect what is improbable. If, however, it can be shown that a certain organism or a material produces an analogous condition leading to the death of the animal, and the post-mortem and microscopical examinations show similar changes, evidence of the cause of the disease in man is afforded.

The production in rats of a condition similar to pellagra.

In September last I obtained two three-quarter grown rats of the same litter; they were placed in the contiguous compartments of the same cage. Their food was prepared in the same vessel, and into one half were stirred various preparations of decomposing corn-meal; this was given each day to one rat, whilst to the other was given the wholesome moiety.

The cage was of wire and was placed in the sun for about two hours each day, so that each rat enjoyed the same amount of sunshine.

By the sixth day of the experiment the condition of the two animals was very different. The one which had taken corn-meal had lost weight, its coat was staring, it was weak in its gait and crouched trembling in the corner of the cage; the consistence of its stools was very soft. The other rat remained normal. The administration of corn-meal preparations was stopped for two days and then recontinued. On the tenth day the bare skin of the ears and legs started peeling and became darker than in the normal rat; this eruption did not reach a very advanced stage, for the rat died on the 14th day. The control animal remained healthy.

Post-mortem. The lungs were normal, but the heart was of an irregular colour.

The liver was in an advanced stage of degeneration, being pale and friable with small patches of blood-red colouration, which appeared to be due to recent haemorrhages. There was not much alteration in the size of the organ, when it was compared with that of the healthy rat which had been killed for comparison.

The spleen was dark coloured, being blackish red; it was smaller and firmer, yet more friable than in the healthy animal.

The kidneys were paler and softer than normal.

The mucosa of the stomach showed one or two petechial haemorrhages.

The intestines were very friable, and there were areas of congestion and numerous haemorrhagic spots throughout their length.

Five other rats were similarly fed, three more being kept as controls:

(No. 1.) A small rat died on the 8th day, and the post-mortem showed a similar condition to that described above.

(No. 2.) This rat, which showed some signs of disease, escaped on the 9th day.

(No. 3.) The third rat died on the 31st day of the experiment, and atrophy, minute haemorrhages, and degeneration of the abdominal viscera were observed at the post-mortem.

(Nos. 4 and 5.) These were killed on the 34th day. One of them was seriously ill, but the other, in comparison, exhibited very few symptoms in life, or pathological changes at the autopsy.

The three control animals had remained healthy.

Microscopic examination of these rats.

The lungs showed no changes. The heart muscle in two cases showed fatty degeneration. In other rats there were haemorrhages between the muscle fibres.

The smaller vessels of the liver showed degeneration, and there were numerous haemorrhages among the liver cells.

Fatty degeneration was present in the glandular cells of this organ.

There was an increase in fibrous tissue in the spleen; numerous haemorrhages had taken place from damage to the arterioles and capillaries. There was a degree of pigmentation present which exactly compared with that which is seen in sections from human subjects who have died of pellagra.

The intestine showed some haemorrhages, but did not exhibit the same degree of degeneration and atrophy which are seen in human cases.

Thus practically all the changes seen in the organs of human pellagrins were present in the organs of these rats; but the advanced arteriole thickening and extreme atrophy and cellular degeneration of chronic human cases were absent.

The corn-meal used was prepared in three ways :

(1) It was boiled with water and the resulting paste allowed to become sour in the laboratory.

(2) Samples were moistened and allowed to remain open in the laboratory.

(3) Cultures of organisms which are present in damaged maize were mixed with moistened corn-meal and incubated for thirty-six hours.

Portions of these preparations were mixed with the food which was given to the rats each day.

These preliminary experiments show that corn-meal acted upon by various micro-organisms develops toxins which may produce in animals a condition analogous to pellagra in man. Though several organisms and active principles can be isolated from soured maize, the exact nature or relation of these to the disease has not yet been worked out.

EXPLANATION OF PLATES IV AND V.

PLATE IV.

Fig. 1. Section of the spleen of a rat which had been fed upon preparations of maize meal. The lower part of the photograph shows masses of red blood cells derived from haemorrhages. Line *A* points to the centre of a light coloured haemorrhagic area.

Fig. 2. A portion of the same section enlarged to show the small haemorrhages among the splenic cells.

Fig. 3. A section of human spleen from a chronic case. Note the general fibrosis and obliteration of small vessels with thickening of the larger arteriole. *A* points to a mass of red blood cells (when highly magnified red blood cells are seen to be very numerous).

Fig. 4. Section of liver showing extreme fatty degeneration; the most advanced condition of this type of degeneration met with in the series of human cases.

PLATE V.

Fig. 5. A section of a rat's liver, in which the parenchymatous structure has been broken up by haemorrhages. Masses of pigment are present, and the liver cells show degeneration.

Fig. 6. A section of the pia mater from a human case; this shows thickening of the small vessels and capillaries. The vessels are stenosed.

Figs. 7, 8. Sections of intestines from two human cases.

A. Points to the atrophic and degenerate mucous membrane. Note the absence of nuclear staining in the villous processes of 7.

B. Points to the submucous coat. In 7 there is a thickened arteriole and to the left of this the coat is swollen by a haemorrhage which has left pigment and degenerate red blood cells. In 8 there is an area of round celled infiltration extending to erosion on the mucous surface.

C. The degenerate and atrophic muscular coats; note the vacuolation present in the fibres.

D. The thickened serous coat. In 7 it has been enormously thickened by a haemorrhage.

These two sections were taken from parts of the small intestine where the changes were most marked.

ON THE INHIBITION OF THE CHOLERA-RED REACTION BY CERTAIN NITRITE-DESTROYING ORGANISMS AND ON THE MUTUAL INHIBITION OF *B. DYSENTERIAE* (FLEXNER) AND *V. CHOLERAÆ* WHEN GROWN TOGETHER.

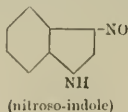
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TOBEY (1908) has published a brief account of a research which he undertook with a view to determining what contaminating organisms interfere with the cholera-red reaction. He found that "four races of *B. coli communis*, *B. pyogenes fetidus*, *B. acidi lactici*, *B. mucosus capsulatus* and *B. enteritidis* Gaertner always prevented the reaction," while *B. typhosus*, *B. faecalis alkaligenes*, *B. icteroides*, *B. dysenteriae* (Flexner) and certain spirilla (Milleri, Deneke, Finkler-Prior) did not affect it. These results have been for the most part confirmed by the present author, and the experiments which follow were undertaken to explain them.

As Pöchl (1886), Bujwid (1888), Dunham (1888) and others have shown a 8-48 hours' growth of *V. cholerae* in 1 per cent. peptone water gives on the addition of a few drops of strong sulphuric acid a pink or "Burgundy wine red" colour. This, as was proved by the researches of Brieger (1887), Salkowski (1887), and Petri (1889), is due to the presence in the culture of both indole and nitrites and to the consequent formation of "nitroso-indole" on the addition of any acid capable of



liberating nitrous acid from its compounds. Obviously the absence of either indole or nitrites is sufficient to abolish the reaction.

In the case of such a mixed growth as that of *B. coli* and *V. cholerae*, indole is not likely to be absent since both organisms produce it. On the other hand nitrites may be absent since *B. coli*, *B. enteritidis* Gaertner, and a large number of other organisms destroy nitrites. This may be shown to be the cause of the failure of the cholera-red reaction in mixed growths in several ways. In the first place, two similar amounts of the same medium may be inoculated with both *B. coli* and *V. cholerae*, and after these cultures have been incubated for 24 hours at 37° C., a small quantity of nitrite may be added to one of them, and then both may be at once tested with sulphuric acid. It will be found that the culture to which nitrite has been added gives the nitrite reaction while the other does not. By a somewhat different method the following experiment proves the same point. Nine tubes were taken, each containing 5 c.c. peptone water (peptone 1 per cent., sodium chloride 0.5 per cent.) and of these, three were inoculated with *B. coli* alone, three with *V. cholerae* alone, and three with both *B. coli* and *V. cholerae* together. The nine cultures were placed in the same incubator for 24 hours at 37° C. and at the end of that period one tube of each set was tested for nitrite, one for indole and one with sulphuric acid for the cholera-red reaction.

The test used for nitrite was that with α -naphthylamine-acetate and sulphanilic acid, for indole Ehrlich's reagent, p-dimethyl-amido-benzaldehyde, was applied according to Boehme's method, and the cholera-red reaction was tested for in the usual way by adding to the culture a few drops of strong nitrite-free sulphuric acid. The results are presented in the following table :

Results of testing for

	Nitrite	Indole	Cholera-red
<i>B. coli</i>	-	+	-
<i>V. cholerae</i>	+	+	+
<i>B. coli</i> + <i>V. cholerae</i>	-	+	-

It is evident from these results that in the mixed growth as in the pure *B. coli* culture the failure to give a cholera-red reaction is due to the absence of nitrite. That the absence of nitrite is not due to its non-formation may be proved by taking samples of the mixed growth at various stages and testing them for nitrites by means of the delicate test with sulphanilic acid and α -naphthylamine-acetate. At first the

samples, like similar ones from pure growths of *B. coli* or *V. cholerae*, show no development of nitrite, but after a few hours nitrites appear in the culture and steadily increase in amount till a strong reaction is given. The amount of nitrite then rapidly diminishes until finally the culture is nitrite-free. In the case of a mixed growth of *B. coli* and *V. cholerae* the disappearance of nitrite occurs in from 15 to 24 hours.

That many micro-organisms destroy nitrites has of course been known for some considerable time. Not only do certain "de-nitrifying" organisms occur in the soil, where they are of some interest from an agricultural point of view, though their denitrifying power seems to be less in the soil than in fluid cultures, but many pathogenic and other organisms possess the same power. Maassen (1901) investigated 109 organisms with respect to their action on nitrites and nitrates, and of these he found that 85 reduced nitrates to nitrites while only 50 reduced nitrites to ammonia. Many of the organisms which reduced nitrates to nitrites failed to destroy nitrites, while some that could destroy nitrites failed to reduce nitrates. *V. cholerae* of course belongs to the group which reduces nitrates to nitrites but fails to reduce nitrites. The present author (Logie, 1910) investigated fourteen strains of dysentery bacilli and found that of these all but one reduced nitrates to nitrites while only seven destroyed nitrites. The five "Shiga" strains examined failed to reduce nitrites and one of them (obtained from Prof. Neisser) failed even to reduce nitrates. Of the nine mannite-fermenting strains, only two (*B. dysenteriae* Jürgens and one of the Neisser strains) failed to destroy nitrites while all attacked nitrates. *B. dysenteriae* (Jürgens) is remarkable inasmuch as it forms both nitrites and indole and consequently gives the cholera-red reaction.

Since *B. coli communis* and *B. enteritidis* Gaertner possess the power of destroying nitrites it is not remarkable that when grown with *V. cholerae* in peptone water they abolish the cholera-red reaction; but since *B. typhosus*, *B. paratyphosus* (A and B) and many strains of *B. dysenteriae* (Flexner) also possess the power of destroying nitrites, it is surprising to find that they do not likewise abolish the cholera-red reaction. *B. dysenteriae* (Flexner) when grown in peptone water in pure culture reduces all the nitrate naturally present to nitrite and destroys the nitrite in less than 24 hours; yet if it is grown along with *V. cholerae* a cholera-red reaction may still be obtained in a 24 hours' growth. This can only be explained on the supposition that the action of *B. dysenteriae* (Flexner) is in some way inhibited by the presence of *V. cholerae*. As a matter of fact the author has found in

repeated experiments that the total number of both organisms in a mixed growth of *B. dysenteriae* (Flexner) and *V. cholerae* is much less than the number of organisms in the same quantity of a pure *B. dysenteriae* (Flexner) culture. This is strikingly shown in the following experiment.

A series of test-tubes, each containing 5 c.c. of peptone water (peptone 1 per cent., sodium chloride 0.5 per cent.) were prepared in the usual way and of these two were inoculated, the one with *B. dysenteriae* (Flexner), the other with *V. cholerae*. From these after 24 hours' incubation at 37° C., three other tubes were inoculated, one with a loopful of *B. dysenteriae* (Flexner), one with a loopful of *V. cholerae*, and one with a loopful of *B. dysenteriae* and also a loopful of *V. cholerae*. This last mentioned test-tube should therefore have contained to begin with as many organisms as the other two together; at all events it should have had more than either singly. After incubation for 24 hours at 37° C. these cultures were plated on Endo-agar in a dilution of one to ten thousand. The plates were made by pouring sterile Endo-agar into sterile Petri dishes (of 4½ inches diameter) and allowing the agar to cool under sterile filter paper till a firm moistureless surface was produced. This usually occurred in a few hours.

The measured quantity (0.025 c.c.) of diluted culture was discharged from a sterile pipette upon the surface of the agar and smeared by means of a sterile platinum needle over the whole available area. To avoid injury to the organisms from possible contact with the hot wire, the platinum wire was plunged into cold sterile water immediately after sterilisation. The plates dried rapidly so that the lids could be replaced and the capsules inverted as soon as the manipulations were complete. The colonies thus obtained were, of course, all surface growths. The following table gives the counts of six plates (two from each of the three cultures) after they had been incubated at 37° C. for 48 hours:

	<i>B. dys.</i> (Flexner)	<i>V. cholerae</i>	<i>B. dys.</i> (Flex.) + <i>V. cholerae</i>
Plate 1	351	149	32
Plate 2	324	147	73
Total	675	296	105

It will be observed that the pure *B. dysenteriae* (Flexner) culture contains by far the largest number of organisms while the mixed culture contains fewest. Indeed it is always possible to arrange the plates in the order *B. dysenteriae* (Flexner), *V. cholerae* and mixed

growth, without reading the labels at all, judging merely by the number of colonies on the plates. If the Endo-agar be made with peptone water in place of bouillon, *V. cholerae* often refuses to grow and a very striking contrast can be obtained between the numerous colonies on the pure Flexner plates and the scanty growth on those from the mixed culture. It is evident, therefore, that both *B. dysenteriae* (Flexner) and *V. cholerae* are inhibited in their growth when grown together.

The growth on the plates from the mixed culture consists of colonies of both organisms and the colonies can be distinguished by their naked eye appearance. Those of *B. dysenteriae* (Flexner) are more delicate and more sculptured, showing at first a pitted centre in which a papilla develops, which ultimately fills the pit completely so that the colony shows a central peak, from which spurs run out towards the indented margins. There is thus a radial striation. A concentric striation is also distinctly seen, its curves following the indentations of the margin, and the whole colony presents the appearance of a delicately sculptured film. When viewed by transmitted light, the colony shows a dark point at the centre which gives it somewhat the appearance of a flea-bite on Endo-agar plates. *V. cholerae* colonies, on the other hand, present an unpitted convex surface and have smooth circular margins. The centre is granular and appears darker than the marginal portion when viewed by transmitted light, but the dark part occupies a larger area proportionally than the central spot of the Flexner colony and the two colonies can be easily distinguished by this means.

As somewhat intermediate forms occur, it is best, if a differential count is desired, to make a sub-culture from each colony on peptone water and confirm the result by testing the sub-cultures for cholera-red after 24 hours' incubation at 37° C. Such a count was made in the case of the experiment quoted above and the result of enumerating the colonies of the two organisms in the mixed plate is given in the following table:

	<i>B. dysenteriae</i> (Flexner)	<i>V. cholerae</i>
Plate 1	16	16
Plate 2	29	44
Total	45	60

It would thus appear that *B. dysenteriae* (Flexner) is inhibited to a greater extent than *V. cholerae*.

Numerous instances, of course, are known of similar inhibition of one organism by another. In many cases a particular medium is

necessary. Indeed the selective influence of a particular medium is made use of for the isolation of organisms, for example, the well-known case of the use of blood serum for the isolation of *B. diphtheriae*. In the present instance the marked inhibition of the growth of *B. dysenteriae* in peptone solution by the cholera organism is noteworthy in connection with the special suitability of this medium for the isolation of *V. cholerae* from the stools. That it is not an unfavourable medium for *B. dysenteriae* (Flexner) is shown by the large number of organisms in the pure Flexner culture—(about 270 million per c.c.).

In the case of organisms like *B. faecalis alkaligenes* which do not reduce nitrite, one does not of course expect the cholera-red reaction to disappear.

SUMMARY.

1. It has been shown that certain nitrite-destroying organisms when grown along with *V. cholerae* prevent the appearance of the cholera-red reaction. This is not due to the non-formation of nitrite, but to its rapid destruction by the nitrite-destroying organisms.

2. Certain nitrite-destroying organisms fail to prevent the cholera-red reaction.

3. It has been shown in the case of the *B. dysenteriae* (Flexner) that the failure to prevent the cholera-red reaction is due to an inhibition of the growth of both organisms when grown together.

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THE RESISTANCE OF SPORES TO HEATING IN ANHYDROUS FLUIDS SUCH AS GLYCERINE AND SIMILAR SUBSTANCES.

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THE use of glycerine, oil and similar substances finds a constantly increasing application in modern surgery and medicine. Accordingly the adequate sterilisation of these fluids becomes a matter of considerable importance.

In this connection a number of observations carried out by Prof. Dreyer and Dr Ainsley Walker made it clear that if resistant spores are present in anhydrous glycerine or oil they are not destroyed at such temperatures and within such periods of time as are frequently regarded as sufficient for this purpose. The results of their experiments led them to state in regard to the value of heating in oil or glycerine that "in the absence of water the effect obtained will be no more than if the organisms had been heated in dry air for the same length of time" (Dreyer and Walker, 1912, p. 13). They subsequently laid it down that it is "necessary for the sterilisation of these fluids to make use of a temperature of from 150° C. to 170° C. for not less than half an hour" (Dreyer and Walker, 1912, p. 142). The present work was undertaken at their suggestion in order to determine more precisely the exact limits of temperature required for sterilisation, and the influence which the medium of suspension may exert upon the disinfecting action of superheated steam, or on that of heat applied in other forms.

This investigation appeared to be of the greater importance in view of the lack of uniformity in the methods at present employed for the sterilisation of glycerine, oil, liquid paraffin, vaseline and the like, few if any of which fulfil the requirement laid down by Dreyer and Walker.

Thus, I have ascertained from inquiries which I instituted for the purpose that the method now in general use in many important institutions is that described by Martindale and Westcott (1908), and consists in heating the substances concerned for from 10 to 30 minutes at 120° C. to 140° C. Another method (kindly communicated to me by Prof. Pearson) which has been employed in the case of glycerine, is to place it in a flask and heat it in the autoclave for three quarters of an hour at about 108° C. (circa $1\frac{1}{4}$ atmospheres of pressure). Claypool, Vance, Robertson and Field (1910) recommend the sterilisation of oil by heating it for fifteen minutes at 115° C. in the autoclave. Crump (1910) in a long series of experiments on peritoneal adhesions used animal oil "sterilised" by heating on three successive occasions at from 80° C. to 88° C. in tightly sealed bottles; and Blake (1908) sterilised his olive oil by heating at 100° C. for half an hour.

Hot oil again is commonly made use of for the rapid sterilisation of syringes employed in the inoculation of vaccines. The usual method is to draw oil heated to 140° C. in and out of the syringe, which is then regarded as sterile. This is a procedure which only lasts for a fraction of a minute.

From these instances it is clear that there exists no general agreement as to the temperature and length of exposure required to ensure the sterilisation of glycerine and oil, or to secure sterilisation by the application of these substances when heated.

Methods.

In the present experiments spores of *Bacillus subtilis* were employed. The bacillus was grown upon the surface of agar until complete sporulation was obtained. An emulsion of the spores was then prepared in sterile normal saline solution.

By means of a standard loop a drop of the emulsion was spread upon each of a number of sterile cover slips of a given size placed in large sterile Petri dishes. The cover slips were then rapidly dried in an incubator and kept in the Petri dishes ready for use.

In the experiments which follow the cover slips were exposed to heat either in air or after placing them in small sterile sample tubes, containing glycerine, oil, saline solution or other fluid and plugged with cotton wool.

After the exposure they were removed with sterile forceps, washed over with sterile normal saline solution, dropped each into a tube of

sterile bouillon and incubated at 37° C. The culture tubes were of a standard width and contained a standard amount of culture medium in all cases.

The fluids in which the cover slips were heated in different experiments were glycerine, olive oil, normal saline solution, and ordinary peptone bouillon prepared from veal. The glycerine was pure anhydrous glycerine with a boiling point of 290° C., the olive oil was the usual commercial product described in the *British Pharmaceutical Codex* (1911), the saline solution was a 0·9 per cent. solution of sodium chloride in distilled water, which like the bouillon had a boiling point of between 101° C. and 102° C.

In all the experiments the degree of heat employed was registered by means of a standardised maximum thermometer which was so arranged as to be subjected to precisely the same conditions as the cover slips.

In the first series of experiments heating in the autoclave was employed, and the time of exposure was reckoned from the moment when the indicator denoted that the required pressure of superheated steam had been reached, and the gas supply was turned off after this pressure had been maintained for the period desired. It will thus be seen that there was an uncounted period of heating while the pressure was being got up, and again while it was falling back to atmospheric pressure after the supply of heat was cut off. As soon as the pressure had fallen to normal again the autoclave was opened, and the cover slips taken out and treated as already described.

In subsequent series of experiments the effect of heating in air in a carefully regulated and well-jacketed hot air oven was investigated, as well as the effect of heating at atmospheric pressure in various fluids. In all cases the cover slips were only introduced into the hot air or into the tubes of heated fluid, as the case might be, after the desired temperature had been reached and maintained constant for some time. The heating was terminated by the removal of the cover slips from the source of heat.

The effect of boiling cover slips with spores in normal saline solution and in bouillon was investigated by heating the tubes containing these fluids in a water bath whose boiling point had been raised to about 105° C. by the addition of borax and sodium chloride to the water which it contained.

In every experiment the viability of the spores employed was tested by means of suitable control tubes. Moreover for every cover slip

exposed to heat in glycerine or oil a corresponding cover slip was similarly exposed in a tube of normal saline solution and a tube of culture bouillon raised to the boiling point by the method described above in order to control the determination of the thermal death point for spores in aqueous fluids.

All the experiments were made with the same batch of spores except those whose results are shown in Table VII. In this latter case spores from a different and, as will be seen, a more resistant culture were employed, and the results obtained are, therefore, not directly comparable with those shown in the rest of the Tables.

Autoclave experiments.

Tables I, II, III and IV show the results of the experiments carried out with superheated steam in the autoclave.

In Table I heating at $1\frac{1}{4}$ atmospheres (about $108^{\circ}\text{C}.$) in glycerine up to two hours in no case killed the spores on the cover slips, while in normal saline solution 60 per cent. of the cover slips were sterilised by 10 minutes heating, and all the spores were destroyed by heating for 15 minutes or more. In the case of bouillon all the spores were killed within 10 minutes.

Table II shows that on heating at $1\frac{1}{2}$ atmospheres (about $113^{\circ}\text{C}.$) for a period longer than the usual time of sterilising in the autoclave, namely for 30 minutes, *none* of the cover slips in glycerine were rendered sterile. After $1\frac{1}{4}$ hours' exposure 20 per cent. were sterilised in the glycerine, and after two hours 60 per cent. In normal saline solution and in bouillon all the spores are killed within five minutes at this temperature and pressure.

From Table III it is seen that heating at $1\frac{3}{4}$ atmospheres (about $117^{\circ}\text{C}.$) does not sterilise the cover slips in glycerine in half an hour. After one hour's exposure 20 per cent. were sterilised, after $1\frac{1}{4}$ hours 60 per cent., and even after $1\frac{1}{2}$ hours only 80 per cent. of the cover slips had been rendered sterile.

Table IV shows that on heating at 2 atmospheres of steam pressure (about $120^{\circ}\text{C}.$) 20 per cent. of the cover slips were sterilised within 20 minutes. After half an hour 70 per cent. had been sterilised, and in 45 minutes and upwards all the spores had been killed.

Hence it follows that if resistant spores happen to be present in it, glycerine cannot be sterilised in the autoclave except by heating at high pressure for considerable periods of time.

The same facts have been shown to hold for the sterilisation of oil.

Resistance of Spores

TABLE I.

Approximate pressure in atmospheres	Range of actual temperature in degrees Centigrade as shown by maximum thermometer	Time of exposure in minutes	Glycerine				Normal saline solution				Bouillon			
			Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed
14	107.5—108.0	10	10	10	0	0	5	2	3	60	5	0	5	100
	"	15	16	16	0	0	8	0	8	100	8	0	8	100
	"	20	5	5	0	0	3	0	3	100	3	0	3	100
	"	30	10	10	0	0	5	0	5	100	5	0	5	100
	"	40	10	10	0	0	4	0	4	100	4	0	4	100
	"	60	5	5	0	0	2	0	2	100	2	0	2	100
	"	75	5	5	0	0	2	0	2	100	2	0	2	100
	"	90	5	5	0	0	2	0	2	100	2	0	2	100
	"	120	5	5	0	0	3	0	3	100	3	0	3	100

TABLE II.

Approximate pressure in atmospheres	Range of actual temperature in degrees Centigrade as shown by maximum thermometer	Time of exposure in minutes	Glycerine				Normal saline solution				Bouillon			
			Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed
14	112.2—112.7	5	5	5	0	0	3	0	3	100	3	0	3	100
	"	10	5	5	0	0	3	0	3	100	3	0	3	100
	"	30	5	5	0	0	3	0	3	100	3	0	3	100
	"	75	5	4	1	20	3	0	3	100	3	0	3	100
	"	120	10	4	6	60	6	0	6	100	6	0	6	100

TABLE III.

Approximate pressure in atmospheres	Range of actual temperature in degrees Centigrade as shown by maximum thermometer	Time of exposure in minutes	Glycerine				Normal saline solution				Bouillon			
			Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed
14	116—116.6	15	5	5	0	0	3	0	3	100	3	0	3	100
	"	30	5	5	0	0	3	0	3	100	3	0	3	100
	"	60	5	4	1	20	3	0	3	100	3	0	3	100
	"	75	10	4	6	60	6	0	6	100	6	0	6	100
	"	90	10	2	8	80	6	0	6	100	6	0	6	100

TABLE IV.

Approximate pressure in atmospheres	Range of actual temperature in degrees Centigrade as shown by maximum thermometer	Time of exposure in minutes	Glycerine				Normal saline solution				Bonillon			
			Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed
2	119.7—120.5	10	5	5	0	0	3	0	3	100	3	0	3	100
	"	20	15	12	3	20	9	0	9	100	9	0	9	100
	"	30	10	3	7	70	6	0	6	100	6	0	6	100
	"	45	5	0	5	100	3	0	3	100	3	0	3	100
	"	60	5	0	5	100	3	0	3	100	3	0	3	100

Heating under ordinary atmospheric pressure.

Experiments were next made with glycerine heated under ordinary atmospheric pressure in the external air to the same temperature as had been used in the preceding autoclave experiments. The results, controlled by results obtained with boiling saline solution and with boiling bouillon, are given in Table V.

TABLE V.

Time of exposure in minutes	Temperature of the Glycerine in degrees Centigrade	Glycerine				Normal Saline Solution				Bouillon					
		Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed	Temperature of the Saline Solution (approximately) in degrees Centigrade	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed	Temperature (approximately) of the Bouillon in degrees Centigrade	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips in which all the spores were killed
30	107.5-108	4	4	0	0	101.5	5	0	5	100	101.4	5	0	5	100
60	"	4	4	0	0	"	5	0	5	100	"	5	0	5	100
80	"	4	4	0	0	"	5	0	5	100	"	5	0	5	100
90	"	4	4	0	0	"	5	0	5	100	"	5	0	5	100
120	"	4	4	0	0	"	5	0	5	100	"	5	0	5	100
120	"	4	4	0	0	"	5	0	5	100	"	5	0	5	100
120	112-113	4	2	2	50	"	4	0	4	100	"	4	0	4	100
75	116-117	4	4	0	0	"	4	0	4	100	"	3	0	3	100
90	"	4	2	2	50	"	4	0	4	100	"	4	0	4	100
30	112-113	12	9	3	25	"	14	7	7	50	"	14	0	14	100
45	"	8	3	5	63	"	6	1	5	83	"	6	0	6	100
60	"	4	0	4	100	"	3	0	3	100	"	3	0	3	100

In comparing these results with those obtained at the same temperatures in the autoclave it must be remembered that the times recorded for the autoclave experiments ignore the period of time occupied in heating up from 100° C. to the required temperature and pressure at the beginning of the experiment, and the time required for cooling down again to 100° C. at its conclusion before the tubes can be removed from the autoclave. Accordingly something like 15 or 20 minutes must be added to the times recorded in Table V to render the results comparable with those obtained in the autoclave experiments. If this obvious correction were omitted it might be imagined that the Tables in question showed that the heating in the autoclave was more efficient for the sterilisation of glycerine than heating to the same temperature in the external air. But this is not the case.

Thus it is seen that on heating cover slips in glycerine in the external air none of them are rendered sterile in two hours at 108° C., a temperature which corresponds to $1\frac{1}{4}$ atmospheres in the autoclave. On heating for two hours at 113° C. 50 per cent. of the cover slips are sterilised, while in the autoclave 60 per cent. were sterilised at 113° C. ($1\frac{1}{2}$ atmospheres) in a period of two hours, plus the time necessary for the heating up and cooling down again. In $1\frac{1}{2}$ hours at 117° C. 50 per cent. of the cover slips are sterilised, while in the autoclave 80 per cent. were sterilised at 117° C. ($1\frac{3}{4}$ atmospheres) in an hour and a half, to which must be added as before an allowance for the time occupied in heating up and cooling down again. In three quarters of an hour at 120° C. 63 per cent. of the cover slips are sterilised, while in the autoclave 100 per cent. (all) were sterilised at 120° C. (2 atmospheres) in the same time, plus the necessary addition. Within one hour all the spores were killed by heating in glycerine at 120° C.

From these observations it is clearly to be seen that heating in glycerine at a given temperature is equally effective in the external air, as under steam pressure in the autoclave, if due allowance be made for the additional exposure to heat which necessarily occurs during the time required for raising the pressure in the autoclave and for lowering it again at the end of the experiment. Thus for example at 120° C. the results obtained in 45 minutes and 60 minutes respectively under atmospheric pressure are produced in 30 minutes and 45 minutes respectively by autoclaving at 120° C., namely the sterilisation of 60 to 70 per cent. of the cover slips in the first case and of 100 per cent. in the second. Other similar examples will readily be found on comparing the results in Table V with those in Tables II, III and IV.

Experiments in the Hot Air Steriliser.

The next series of experiments were carried out in the hot air steriliser. The results obtained are shown in Table VI.

TABLE VI. *Hot air steriliser.*

Temperature in degrees Centigrade	Time of exposure in minutes	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips sterilised
117	45	5	5	0	0
"	60	5	5	0	0
"	75	5	4	1	20
"	90	10	5	5	50
120	10	5	5	0	0
"	20	5	5	0	0
"	30	15	13	2	13
"	45	10	2	8	80
"	60	5	0	5	100

On comparing Table VI with Table V it is seen that heating spores in dry air in the hot air steriliser has for all practical purposes the same sterilising effect as heating in glycerine at the same temperature for the same length of time. This fact is brought out in the following table.

TABLE VII.

Temperature in degrees Centigrade	Time of exposure in minutes	Percentage of cover slips sterilised when exposed in	
		Glycerine	Hot air
117	60	0	0
	75	0	20
	90	50	50
120	20	0	0
	30	25	13
	45	63	80
	60	100	100

It will be noted that the foregoing table shows a complete sterilisation of the cover slips within one hour at 120° C. But it must not be supposed that this degree of heating is to be regarded as likely to be adequate for the destruction of spores in general, since with different strains of spores of the *Bacillus subtilis*, Dreyer and Walker found that heating to as much as 170° C. for half an hour might be necessary to secure absolute sterilisation. A similarly high resistance was exhibited by a batch of *B. subtilis* spores which I prepared during the later part

of the present investigation. This is shown in Table VIII where none of the cover slips were sterilised within an hour and a half at 150° C. in the hot air steriliser. They were however all killed at this temperature in a little over two hours, and were destroyed by an exposure to 180° C. for as short a period even as ten minutes.

Thus although Dreyer and Ainley Walker were content to recommend heating at from 150° to 170° C. for at least half an hour, the results of my detailed experiments seem to show that it is desirable to increase the requirement and to employ a temperature not below 170° C. for at least half an hour or a temperature of 180° C. for from 10 to 15 minutes.

TABLE VIII. *Hot air steriliser.*

Temperature in degrees Centigrade	Time of exposure in minutes	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips sterilised
150	90	6	6	0	0
	100	6	3	3	50
	120	6	1	5	83
	130	6	0	6	100
	140	3	0	3	100
	150	3	0	3	100
180	6	10	5	5	50
	10	2	0	2	100
	15	2	0	2	100
	20	4	0	4	100
	30	10	0	10	100
	35	8	0	8	100
	40	4	0	4	100

TABLE IX.

Time of exposure in minutes	Normal Saline Solution					Bouillon				
	Temperature (approximately) in degrees Centigrade of the Saline Solution	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips sterilised	Temperature (approximately) in degrees Centigrade of the Bouillon	Number of cover slips exposed	Number of cover slips from which growth was obtained	Number of cover slips from which no growth was obtained	Percentage of cover slips sterilised
10	101.5	3	2	1	33	101.4	3	2	1	33
20	"	9	6	3	33	"	9	2	7	78
30	"	19	7	12	63	"	19	0	19	100
45	"	6	1	5	83	"	6	0	6	100
60	"	8	0	8	100	"	8	0	8	100
90	"	5	0	5	100	"	5	0	5	100

An interesting and somewhat curious fact emerges from a comparison of the various control experiments made by boiling spores in normal saline solution and in ordinary culture bouillon respectively. For it is seen that although the boiling point of the bouillon is approximately the same as that of the saline solution employed the percentage of cover slips sterilised by boiling within a given time is considerably greater in the case of the bouillon than in the case of the saline solution. This is shown in Table IX and there is an indication of a difference of the same kind in the first autoclave experiment in Table I.

CONCLUSIONS.

1. In the sterilisation of glycerine (or oil) the use of the autoclave is without special value, since the exposure of spores suspended in glycerine (or oil) to superheated steam acts no more rapidly or effectively than simple heating of these fluids to the same temperature at the ordinary atmospheric pressure.

2. The heating of spores in glycerine (or oil) has no greater sterilising action than simply heating them in dry air in the hot air steriliser at the same temperature for the same period of time.

3. For the sterilisation of these fluids it is necessary to use a temperature of not less than 170°C . for at least half an hour, or a temperature of 180°C . for not less than from ten minutes to fifteen minutes.

4. The methods commonly in use for the sterilisation of glycerine and similar fluids are quite inadequate to ensure sterility with certainty, and accordingly they ought to be abandoned.

In conclusion I wish to express my best thanks to Prof. Dreyer and Dr Ainley Walker for constant help and advice during my work in the Department of Pathology at Oxford.

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STUDIES IN THE MEANING AND RELATIONSHIPS
OF BIRTH AND DEATH RATES. I. THE RELATIONSHIP
BETWEEN "CORRECTED" DEATH
RATES AND LIFE TABLE DEATH RATES.

By JOHN BROWNLEE, M.D., D.Sc.

(With 2 Diagrams.)

CIRCUMSTANCES in connection with the recent census have again directed my attention to the laws which govern human life. I have long been of the opinion that the old ideas that birth rates and death rates had no biological relationship beyond the obvious ones, that many infants mean more deaths, etc., usually found stated in public health text books, were based on a very imperfect induction. On one aspect of this I published a paper a number of years ago in the Transactions of the Royal Philosophical Society of Glasgow⁽¹⁾, and last summer Sir Shirley Murphy⁽²⁾ read a paper on another aspect of the same subject before the Sanitary Congress held at York. But I have hitherto refrained from publishing theories because I believe that until quantitative measures are applied no scientific results worthy of discussion can be obtained. Now that such seem to be possible, I propose to discuss in a series of papers the different relationships which I have investigated. No mathematics will be introduced in the earlier papers, but the results of all will be summarised and dealt with in their mathematical and physico-chemical relationships in a concluding communication. The first paper relates to the connection between the "corrected" death rates and the "true" death rates as found by constructing a life table.

Many conclusions in public health work depend on the use of death rates as a means of comparing the state of health in different districts. The death rate commonly used is termed the "crude" death rate, and is obtained by dividing the total number of deaths in a district in one year

by the total number of inhabitants. The result is given in parts per thousand. This criterion has long been known to be of doubtful worth. The mortality varies with age and sex, and even in adjacent districts the distribution of persons according to these categories is so different as to preclude the comparison. Thus towns contain a very large number of young adults attracted by the opportunity of obtaining work, and as these constitute the healthiest part of the community the "crude" death rate is correspondingly reduced. To meet this, the refinement of the "corrected" death rate has been introduced. The mortalities for each sex at each age period having been ascertained, these are applied severally to the different age and sex groups of the population in the whole country (termed in this connection a "standard population"). This gives the figure which would be found if the mortalities in the district could be assumed to hold for the whole country. Such figures obviously admit of more certain comparison among themselves than those obtained by the older method. The drawback to this method is evidently the fact that the standard population is not a stationary but an increasing population, in which there is, more or less consistently, a smaller number of persons living as age is approached than would be present in a population in which the death rate equals the birth rate. More infants exist, it is true, than in a stationary population, but the period of the high mortality in childhood is short, not more than five years, and the next few quinquennia have a very low mortality, while at the later ages where the mortality is high there are relatively fewer persons living. Thus a "corrected" death rate is not a real death rate. It may be fictitiously low. This point is very important since many conclusions are daily being drawn from such figures. We hear of garden cities with mortalities of 7 per 1000, etc., though even 12 per 1000 is a death rate to be interpreted only with knowledge and discrimination. Neither, I fancy, can under the best conceivable conditions have any real meaning. This is obvious when we consider that in a stationary population the average age of the population in years at death or the expectation at birth is obtained by dividing the number of persons living by the number of deaths per annum. Thus if the population be 1000 and the annual number of deaths 20, the average age at death is 50 years, a possible result. Twelve per 1000 means the average age of 83 years, 7 per 1000 an average age of 143 years, both sufficiently ridiculous. The usual way to attain the truth is to construct a life table, but that is a process requiring both labour and mathematical skill. Were this the only solution it would be well-nigh impracticable

to press its use, but a considerable number of life tables have now been calculated, and by the use of these the true death rate may easily be estimated if the "corrected" death rate be known.

The life tables utilised in the calculations made for this paper are given in the following table. For convenience each is hereafter referred to by the letter placed opposite its description.

TABLE I.

Life Table of England (Farr) 1838-1851	E ₁
" " " (Ogle) 1871-1881	E ₂
" " " (Tatham) 1881-1891	E ₃
" " " (Tatham) 1891-1901	E ₄
Healthy District Life Table of England (Farr) 1849-1851	...	F	
" " " " " (Tatham) 1881-1890	...	H ₁	
" " " " " (Tatham) 1891-1901	...	H ₂	
Brighton Life Table (Newsholme) 1881-1890	...	B	
Manchester Life Table (Tatham) 1881-1890	...	M	
London Life Table (Murphy) 1891-1900	...	L	
Scottish Life Table (Adam) 1891-1900	...	S	
Glasgow Life Table (Chalmers) 1891-1900	...	G	

The number of tables is twelve. The first eleven give in all respects absolutely concordant results. The last, that for Glasgow, shows some differences, due I think to the fact that all the deaths at high ages belonging to Glasgow are not included. At the period for which it was constructed there was no mechanism by which deaths occurring in institutions outside Glasgow could be returned to the city, and the number of institutions outside Glasgow was very considerable. This criticism is borne out by the results of the recent census, and by a comparison with the death rates in Glasgow at the present time.

A life table as usually understood may be defined as the numerical construction of a stable population which possesses the same mortalities at each age as those in the population to be examined. By this means irregularities in the proportions of persons of different ages and sexes, due to varying birth rates and to immigration and emigration, are eliminated. The death rate of any population either "crude" or "corrected" will not be that of the life table. Generally it will be below the latter. Exceptionally, if the death rate is sufficiently high as to annul the natural increase and bring about a stationary or a declining population the "crude" or "corrected" death rate is found to be equal or greater than that obtained when a life table has been constructed.

Two examples will illustrate this. The corrected death rate of the males in the healthy district life table H_2 is found to be 13.49. The actual expectation of life is 52.87 years, giving a death rate of 18.91, both quite conceivable figures. A death rate of 13.49 gives, however, on a stationary population a mean life of $\frac{1000}{30.49}$ or 7.4 years, a figure quite impossible. Manchester on the other hand has a corrected death rate of 28 per 1000. The expectation of life is 34.71 years, giving a death rate of 28.81 on a stationary population. In other words, we do not really have variations of the death rate from 13.5 to 28, but from 18.9 to 28.8, a difference much more easily understood.

For certain reasons, which will be discussed in a later paper, I think that the very highest mean age possible is about sixty years, and this represents a real death rate of 16.3.

How then is this true or life table death rate to be obtained? The method, which partly depends on the biological response of mankind to unhealthy conditions and is partly a pure arithmetical necessity, is expressed in the statement that the relationship between the true death rate and the corrected death rate is linear; that is, given the latter, the former is obtained by multiplying by a constant fraction and adding a definite constant. Thus if D_2 be the real death rate for the whole population of a district and D_1 the "corrected" death rate,

$$D_2 = .6842D_1 + 9.65.$$

D_2 is thus equal to D_1 when both are equal to 30.5. For diagrammatic purposes the difference between the true and the corrected death rates is the better figure to choose, in which case the above formula may be written

$$D_2 - D_1 = -.3158D_1 + 9.65.$$

But the theorem is yet more general. It is not necessary to begin at birth, any age is equally appropriate. To obtain the expectations of life at each period, all that it is necessary to know is the "corrected" death rate for all persons above that age.

These are severally calculated in exactly the same manner as the "corrected" death rate itself is calculated. The multiplications are the same, the only difference being that the sum is made by stages. Thus the two products at 75— and 65—75 are added together, then the product at 55—65 to the latter sum, and so on, so that we have a series of sums each to be divided by corresponding numbers obtained by summing the standard population in the same way. Corresponding to this series of death rates we have from the life tables a similar series of

real death rate figures obtained by dividing 1000 by the expectation of life at the corresponding ages. At each age the relationship between the two series of figures is linear, the general equations, which are equivalent, being

$$D_2 - D_1 = -m D_1 + C$$

and

$$D_2 = (1 - m)D_1 + C.$$

The series of values of m and C from 0 to 55 years is given for both sexes in the accompanying table (Table II).

TABLE II.

Age	Males			Females		
	m	$1 - m$	C	m	$1 - m$	C
0	·3188	·6811	9·54	·3151	·6849	9·32
5	·5141	·4859	12·68	·5216	·4784	12·05
10	·5414	·4586	13·49	·5670	·4330	13·36
15	·4868	·5132	13·53	·5666	·4334	14·35
20	·5140	·4860	14·95	·5400	·4600	15·06
25	·4883	·5117	15·74	·5246	·4754	15·98
35	·4953	·5047	19·16	·4922	·5078	18·05
45	·4953	·5047	23·63	·4397	·5603	20·54
55	·3052	·6948	20·18	·3575	·6425	22·66

The data on which these figures are based are given in Table III. In parallel columns the corrected death rate D_1 the true death rate D_2 obtained from the life table, the actual difference being between the true death rate and the life table death rate, $D_2 - D_1$, and the theoretical value of the latter obtained by fitting the best straight lines, either by the method of least squares or by inspection, are given. The differences found between the real and the theoretical values are tabulated next, and the value of the square root of the mean of the squares of these differences, denoted by Δ , is added. This last figure gives the measure of the difference between the real and theoretical values. It rarely exceeds one per cent. The relationship however is best seen in diagrams. For this purpose the male death rates at 15 years (Diagram I) and at 55 years (Diagram II) have been chosen. Both illustrate well the concordance of fact and theory, and though the divergence is greater in the latter case than in the former the relationship is obviously truly linear.

When the great range of the corrected death rates is considered, *e.g.*, at birth in the case of males, from 13 per 1000 to 28 per 1000, this must be considered a very small error in a prediction of the true death

rate. In fact the values obtained by the method here given may with some confidence be taken as probably more accurate than those obtained directly by constructing a life table, inasmuch as they represent the average of many life tables. The figures of one life table are at best

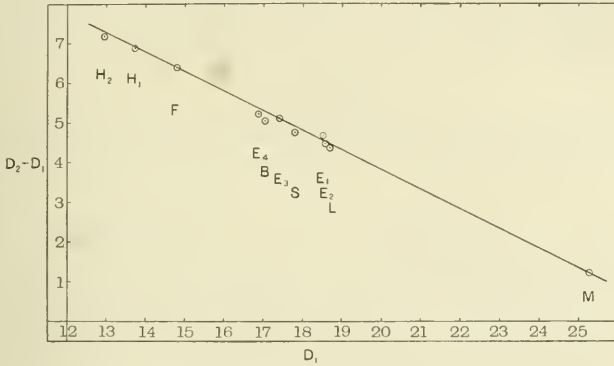


Diagram I.

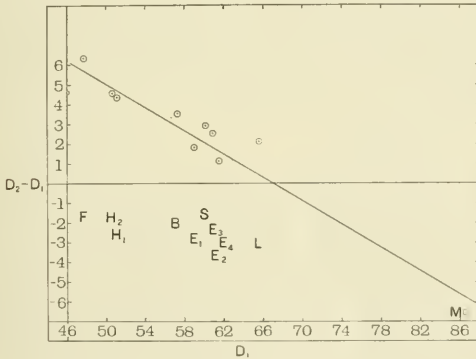


Diagram II.

but a close approximation. So much immigration at certain ages takes place from country to town *i.e.*, from healthy to unhealthy conditions, that the average death rates at the ages of migration represent not one

phenomenon but a mixture of phenomena. The same is more or less true at all ages, since methods of living vary from class to class and all classes are grouped together in the one table. The error due to these factors, however, cannot be great on the whole, but may, as the above tables seem to show, be taken as probably not more than one per cent. That special populations such as Brighton should show some differences at high ages is not more than would be naturally expected, since the

TABLE III.

Showing the values of D_2 and D_1 obtained from the Life Tables and from the "standard" population and their relationships.

Age 0. Males.						Age 0. Females.					
	D_1	D_2	$\frac{D_2-D_1}{\text{Act.}}$	$\frac{D_2-D_1}{\text{Theor.}}$	Diff.		D_1	D_2	$\frac{D_2-D_1}{\text{Act.}}$	$\frac{D_2-D_1}{\text{Theor.}}$	Diff.
H ₂	13.49	18.91	5.42	5.29	-.13	12.49	17.95	5.46	5.38	-.08	
H ₁	14.26	19.42	5.16	5.05	-.11	13.40	18.50	5.10	5.10	.00	
F	16.03	20.59	4.56	4.49	-.13	15.95	20.22	4.27	4.29	+.02	
E ₄	19.32	22.66	3.34	3.45	+.11	17.14	20.93	3.79	3.92	+.13	
E ₃	19.79	22.90	3.11	3.30	+.19	17.74	21.19	3.45	3.73	+.28	
E ₂	21.61	24.18	2.54	2.72	+.18	19.40	22.41	3.01	3.36	+.35	
E ₁	22.30	25.06	2.76	2.51	+.05	21.00	23.90	2.90	2.90	.00	
B	19.75	22.94	3.19	3.31	+.12	16.05	20.41	4.36	4.26	-.10	
S	19.21	22.36	3.15	3.58	+.43	17.31	21.06	3.75	3.88	-.13	
L	21.82	24.40	2.58	2.66	+.08	18.49	22.06	3.57	3.49	-.08	
M	28.00	28.81	0.81	0.91	+.10	24.46	26.02	1.56	1.64	+.08	
G	26.43	28.43	2.00	1.20	-.80	24.15	26.52	2.37	1.71	-.66	
Δ (excluding G)					.17						.15
$D_2 = .6811D_1 + 9.54.$						$D_2 = .6849D_1 + 9.32.$					
Age 5. Males.						Age 5. Females.					
	D_1	D_2	$\frac{D_2-D_1}{\text{Act.}}$	$\frac{D_2-D_1}{\text{Theor.}}$	Diff.		D_1	D_2	$\frac{D_2-D_1}{\text{Act.}}$	$\frac{D_2-D_1}{\text{Theor.}}$	Diff.
H ₂	10.29	17.16	6.87	7.08	+.21	10.11	16.80	6.69	6.74	+.05	
H ₁	10.99	17.53	6.54	6.70	+.16	10.92	17.24	6.32	6.32	.00	
F	12.21	18.39	6.18	6.04	-.11	13.18	18.54	5.36	5.13	-.23	
E ₄	13.41	18.69	5.28	5.38	+.10	12.50	17.92	5.42	5.49	+.07	
E ₃	13.98	18.96	4.98	5.07	+.09	13.29	18.21	4.92	5.07	+.15	
E ₂	15.14	19.66	4.52	4.44	-.08	14.32	18.84	4.52	4.53	+.01	
E ₁	15.58	20.12	4.54	4.20	-.34	15.94	19.87	3.93	3.68	-.25	
B	13.57	18.54	4.97	5.30	+.33	11.29	17.57	6.28	6.12	-.16	
S	14.25	19.10	4.85	4.93	+.08	13.38	18.51	5.13	5.12	-.01	
L	14.85	19.38	4.53	4.60	+.07	12.85	18.18	5.33	5.31	-.02	
M	20.22	21.93	1.71	1.68	-.03	18.43	20.81	2.38	2.38	.00	
Δ					.18						.13
$D_2 = .4559D_1 + 12.68.$						$D_2 = .4754D_1 + 12.05.$					

Age 10. Males.

	D_1	D_2	$D_2 - D_1$ Act.	$D_2 - D_1$ Theor.	Diff.
H ₂	11.37	18.46	7.09	7.33	+ .24
H ₁	12.07	18.84	6.77	6.96	+ .19
F	13.04	19.50	6.46	6.43	+ .03
E ₄	14.79	20.15	5.36	5.48	+ .12
E ₃	15.29	20.49	5.20	5.21	+ .01
E ₂	16.42	21.09	4.67	4.60	- .07
E ₁	16.56	21.26	4.70	4.53	- .17
B	14.90	20.09	5.19	5.42	+ .23
S	15.69	20.58	4.89	5.00	+ .11
L	16.38	20.90	4.52	4.62	+ .10
M	22.13	23.39	1.26	1.51	+ .25
Δ					.16

$$D_2 = .4586D_1 + 13.49.$$

Age 15. Males.

	D_1	D_2	$D_2 - D_1$ Act.	$D_2 - D_1$ Theor.	Diff.
H ₂	12.97	20.13	7.16	7.22	+ .06
H ₁	13.73	20.57	6.84	6.85	+ .01
F	14.80	21.19	6.39	6.33	- .06
E ₄	16.88	22.12	5.24	5.31	+ .07
E ₃	17.38	22.49	5.11	5.06	- .05
E ₂	18.57	23.05	4.48	4.49	+ .01
E ₁	18.50	23.16	4.66	4.52	- .14
B	17.03	22.08	5.05	5.24	+ .19
S	17.79	22.55	4.76	4.87	+ .11
L	18.68	23.04	4.36	4.44	+ .08
M	25.26	26.47	1.21	1.23	+ .02
Δ					.09

$$D_2 = .5132D_1 + 13.53.$$

Age 20. Males.

	D_1	D_2	$D_2 - D_1$ Act.	$D_2 - D_1$ Theor.	Diff.
H ₂	14.85	22.04	7.19	7.31	+ .12
H ₁	15.68	22.52	6.84	6.89	+ .05
F
E ₄	19.34	24.38	5.04	5.00	- .04
E ₃	19.84	24.83	4.99	4.75	- .24
E ₂	21.08	25.38	4.30	4.11	- .19
E ₁	20.65	25.33	4.68	4.34	- .34
B	19.46	24.34	4.88	4.95	+ .07
S	19.60	24.73	5.13	4.88	- .25
L	21.50	25.56	4.06	3.90	- .16
M	28.99	28.90	- .09	- .05	+ .04
Δ					.17

$$D_2 = .4860D_1 + 14.95.$$

Age 10. Females.

	D_1	D_2	$D_2 - D_1$ Act.	$D_2 - D_1$ Theor.	Diff.
H ₂	11.08	18.03	6.95	7.16	+ .21
H ₁	11.92	18.52	6.60	6.60	.00
F	14.04	19.65	5.61	5.40	- .21
E ₄	13.64	19.24	5.60	5.62	+ .02
E ₃	14.42	19.57	5.15	5.18	+ .03
E ₂	15.46	20.10	4.64	4.59	- .05
E ₁	16.93	20.97	4.04	3.76	- .28
B	12.25	18.81	6.56	6.41	- .15
S	14.53	19.85	5.32	5.12	- .20
L	13.92	19.42	5.50	5.46	- .04
M	19.98	22.01	2.03	2.03	.00
Δ					.17

$$D_2 = .4330D_1 + 13.36.$$

Age 15. Females.

	D_1	D_2	$D_2 - D_1$ Act.	$D_2 - D_1$ Theor.	Diff.
H ₂	12.44	19.58	7.14	7.30	+ .16
H ₁	13.34	20.13	6.79	6.79	.00
F	15.47	21.25	5.78	5.58	- .20
E ₄	15.35	21.00	5.65	5.65	.00
E ₃	16.17	21.48	5.31	5.18	- .13
E ₂	17.27	21.92	4.65	4.56	- .09
E ₁	18.71	22.78	4.07	3.75	- .32
B	13.75	20.18	6.43	6.55	+ .12
S	16.21	21.62	5.41	5.15	- .26
L	15.68	21.23	5.55	5.46	- .09
M	22.50	24.10	1.60	1.60	.00
Δ					.16

$$D_2 = .4334D_1 + 14.35.$$

Age 20. Females.

	D_1	D_2	$D_2 - D_1$ Act.	$D_2 - D_1$ Theor.	Diff.
H ₂	13.97	21.31	7.34	7.51	+ .17
H ₁	14.91	21.92	7.01	7.01	.00
F
E ₄	17.36	23.02	5.68	5.69	+ .01
E ₃	18.18	23.57	5.39	5.24	- .15
E ₂	19.30	24.00	4.70	4.63	- .07
E ₁	20.57	24.82	4.25	3.95	- .30
B	15.61	22.34	6.73	6.63	- .10
S	18.11	23.58	5.47	5.28	- .19
L	17.85	23.38	5.53	5.41	- .12
M	25.52	26.79	1.27	1.27	.00
Δ					.13

$$D_2 = .4600D_1 + 15.06.$$

Birth and Death Rates

Age 25. Males.						Age 25. Females.					
	D_1	D_2	$D_2 - D_1$ Act.	$D_2 - D_1$ Theor.	Diff.		D_1	D_2	$D_2 - D_1$ Act.	$D_2 - D_1$ Theor.	Diff.
H ₂	16.94	24.20	7.26	7.47	+ .21		15.94	23.33	7.39	7.61	+ .22
H ₁	17.77	24.76	6.99	7.06	+ .07		16.83	23.98	7.15	7.15	.00
F	17.88	24.76	6.88	7.01	+ .13		18.65	24.89	6.24	6.29	+ .05
E ₄	22.19	27.02	4.83	4.90	+ .07		19.94	25.40	5.46	5.51	+ .05
E ₃	22.66	27.56	4.90	4.68	-.22		20.72	25.97	5.25	5.10	-.15
E ₂	23.82	28.02	4.20	4.11	-.09		21.81	26.33	4.52	4.53	+ .01
E ₁	22.87	27.72	4.85	4.57	-.28		22.87	26.99	4.12	3.98	-.14
B	22.33	26.94	4.61	4.81	+ .23		18.06	24.70	6.64	6.50	-.14
S	22.14	27.21	5.07	4.93	-.14		20.53	25.89	5.36	5.20	-.16
L	24.82	28.60	3.78	3.62	-.16		20.72	26.00	5.28	5.11	-.17
M	33.38	32.58	-.80	-.56	+ .24		29.41	29.96	.55	.55	.00
Δ					.18						.12
$D_2 = .5117D_1 + 15.74.$						$D_2 = .4754D_1 + 15.98.$					
Age 35. Males.						Age 35. Females.					
	D_1	D_2	$D_2 - D_1$ Act.	$D_2 - D_1$ Theor.	Diff.		D_1	D_2	$D_2 - D_1$ Act.	$D_2 - D_1$ Theor.	Diff.
H ₂	22.78	30.00	7.22	7.88	+ .66		21.40	28.74	7.34	7.51	+ .17
H ₁	23.63	30.58	6.95	7.46	+ .51		22.12	29.28	7.16	7.16	.00
F	22.76	30.58	7.82	7.89	+ .07		23.49	29.88	6.39	6.49	+ .10
E ₄	29.96	34.20	4.24	4.32	+ .08		26.84	31.73	4.89	4.83	-.06
E ₃	30.15	34.59	4.44	4.23	-.21		27.37	32.09	4.72	4.57	-.15
E ₂	31.11	34.92	3.81	3.75	-.06		28.39	32.36	3.97	4.07	+ .10
E ₁	29.39	34.01	4.62	4.60	-.02		28.53	32.69	4.16	4.00	-.16
B	29.69	33.96	4.27	4.15	+ .18		24.35	30.79	6.44	6.06	-.38
S	29.41	34.13	4.72	4.59	-.13		26.88	31.87	4.99	4.81	-.18
L	33.51	36.70	3.19	2.56	-.63		28.22	32.87	4.65	4.16	-.49
M	44.64	42.09	-2.55	-2.95	-.40		39.33	38.02	-1.31	-1.31	.00
Δ					.35						.22
$D_2 = .5047D_1 + 19.16.$						$D_2 = .5078D_1 + 18.05.$					
Age 45. Males.						Age 45. Males.					
	D_1	D_2	$D_2 - D_1$ Act.	$D_2 - D_1$ Theor.	Diff.		D_1	D_2	$D_2 - D_1$ Act.	$D_2 - D_1$ Theor.	Diff.
H ₂	32.59	39.23	6.64	7.49	+ .85		30.16	37.37	7.21	7.28	+ .07
H ₁	32.28	39.70	7.42	7.64	+ .22		30.53	37.65	7.12	7.12	.00
F	31.38	39.14	7.76	8.09	+ .33		31.52	37.79	6.27	6.63	+ .41
E ₄	41.77	45.05	3.28	2.94	-.34		37.10	41.32	4.22	4.23	+ .01
E ₃	41.50	45.34	3.84	3.08	-.76		37.33	41.58	4.25	4.13	-.12
E ₂	42.17	45.31	3.14	2.74	-.40		38.34	41.57	3.23	3.68	+ .45
E ₁	39.96	42.09	2.13	3.84	+ 1.71		37.81	41.57	3.76	3.92	+ .16
B	40.40	44.37	3.97	3.62	-.35		33.46	39.89	6.43	5.83	-.60
S	40.90	44.97	4.07	3.37	-.70		36.66	41.20	4.54	4.42	-.12
L	45.83	48.43	2.60	.96	-1.64		38.66	42.93	3.27	3.54	+ .27
M	60.67	53.19	-7.48	-6.42	+ 1.06		53.52	50.53	-2.99	-2.99	.00
Δ					.91						.24
$D_2 = .5047D_1 + 23.63.$						$D_2 = .5603D_1 + 20.54.$					

Age 55. Males.						Age 55. Females.					
	D_1	D_2	D_2-D_1 Act.	D_2-D_1 Theor.	Diff.		D_1	D_2	D_2-D_1 Act.	D_2-D_1 Theor.	Diff.
H ₂	50.57	55.19	4.62	5.25	+ .63	46.14	52.30	6.16	6.16		.00
H ₁	51.25	55.56	4.31	5.04	+ .73	46.26	52.47	6.21	6.12		-.09
F	47.74	54.08	6.34	6.11	-.23	47.71	51.97	4.26	5.60	+ 1.34	
E ₄	61.79	63.33	1.54	1.82	+ .28	54.82	58.00	3.18	3.05		-.13
E ₃	60.93	63.53	2.60	2.08	-.52	54.95	58.04	3.09	3.01		-.08
E ₂	61.57	62.70	1.13	1.89	+ .76	56.35	57.70	1.35	2.50	+ 1.15	
E ₁	59.04	60.89	1.85	2.66	+ .81	56.65	57.37	0.72	2.40	+ 1.68	
B	57.28	60.83	3.55	3.20	-.35	48.85	54.11	5.26	5.19		-.07
S	60.10	63.09	2.99	2.34	-.65	53.66	57.40	3.74	3.47		-.27
L	65.59	67.75	2.16	.66	- 1.50	55.91	59.81	3.90	2.67	- 1.23	
M	86.59	80.06	- 6.53	- 5.75	+ .78	76.63	71.89	- 4.74	- 4.74		.00
Δ					.72						.83
$D_2 = .6948D_1 + 20.18.$						$D_2 = .6425D_1 + 22.66.$					

numbers on which the life table is based at these ages are not large enough to permit of certain conclusions. The result of the above investigation is in accordance with the view that the figures on which the Glasgow life table was based were probably not quite trustworthy. Had the life table for Scotland as a whole agreed with that of Glasgow we might have surmised that different conditions held in the two countries of England and Scotland, but the latter falls into line with the other English life tables. The other possibility, that the large numbers of Irish extraction present in Glasgow, approximately one-sixth to one-fourth of the whole population, have had a disturbing effect on the local death rates is of course open to consideration, but the absence of any life table for Ireland itself leaves us without the appropriate data to determine whether the latter hypothesis will bear examination.

In conclusion a few notes are necessary regarding the exact method in which the calculations discussed in the previous portion of the paper should be made. If only the "true" male and "true" female death rate is desired, all that is required is to calculate the corrected death rate for males and females in the usual way, using the proportionate population of England in the years 1891 to 1900 on account of the fact that all the constants of the "corrected" death rates in the above columns have been calculated on these figures. This gives at once the "true" death rates, with a probable error of not more than one per cent.

In order to facilitate the working of the complete method, all the figures necessary for its application are given in Table IV, namely, the proportionate age distribution in the population of England from 1891 to 1900 for males and females, and in parallel columns the

TABLE IV.

Showing the proportions of each sex in the standard population, namely England 1891-1900 and in parallel columns the sums from each age upwards.

Numbers in standard population			Sums from each age upwards		
Age period	Males	Females	Age	Males	Females
0- 5	59052	59468	0-	484057	515943
5-10	56000	56289	5-	425095	456475
10-15	53521	53550	10-	369005	400186
15-20	49986	50814	15-	315484	346636
20-25	44106	49419	20-	265498	295822
25-35	74159	81938	25-	221392	246403
35-45	57412	61276	35-	147233	164465
45-55	41980	45629	45-	89821	103189
55-65	27212	31184	55-	47841	57560
65-75	15026	18596	65-	20629	26376
75-	5603	7780	75-	5603	7780

TABLE V.

Table showing the calculation of Life Table for Liverpool Registration District 1891-1900.

Males.						
Age period	Death rates	Age	Sum products above each age	Corrected death rates above each age	True death rates	Expectation of life in years
0- 5	121.49	0-	18332245	37.872	35.31	28.30
5-10	9.42	5-	11158018	26.253	24.65	40.56
10-15	4.64	10-	10630498	28.808	26.70	37.45
15-20	7.43	15-	10382160	32.908	30.42	32.87
20-25	9.78	20-	10010764	37.705	33.28	30.05
25-35	16.63	25-	9579407	43.268	37.88	25.72
35-45	28.89	35-	8346145	56.686	47.77	20.93
45-55	44.95	45-	6687512	74.453	61.20	16.34
55-65	71.00	55-	4800511	100.34	89.90	11.12
Females.						
0- 5	107.67	0-	17049658	33.015	31.95	31.30
5-10	8.40	5-	10646738	23.323	23.13	43.25
10-15	4.16	10-	10173910	25.422	24.37	41.04
15-20	5.04	15-	9951142	28.703	26.79	37.30
20-25	6.73	20-	9695040	32.773	30.13	33.19
25-35	13.05	25-	9362450	37.996	34.04	29.39
35-45	24.73	35-	8293159	50.425	43.66	22.90
45-55	38.70	45-	6777804	65.683	57.34	17.44
55-65	60.20	55-	5011961	87.073	78.60	12.72

sum of these figures from birth and any age thereafter up to old age. To illustrate the method the expectations of life at ages 0-55 are calculated for the registration district of Liverpool for the same period. The process is shown in Table V. The death rates at each age for both sexes are given in the first column, next follow the sum of these products from each definite age upwards. Parallel to these are the corrected death rates at each age from 0 to 55, obtained by

TABLE VI.

Expectation of life at different ages.

Males.											
	H ₂	H ₁	F	E ₄	E ₃	E ₂	E ₁	B	S	L	M
0	52·87	51·48	48·56	44·13	43·66	41·35	39·91	43·59	44·71	40·98	34·71
5	58·26	57·05	54·39	53·50	52·75	50·87	49·71	52·87	52·36	51·60	45·59
10	54·16	53·07	51·28	49·63	49·00	47·60	47·05	49·12	48·60	47·84	42·75
15	49·67	48·62	47·20	45·21	44·47	43·41	43·18	44·67	44·34	43·40	38·78
20	45·37	44·41	43·40	41·02	40·27	39·40	39·48	40·55	40·43	39·13	34·62
25	41·32	40·39	39·93	37·01	36·28	35·68	36·12	36·51	36·75	34·96	30·69
35	33·32	32·70	32·90	29·24	28·91	28·64	29·40	29·02	29·30	27·25	23·76
45	25·49	25·19	25·65	22·20	22·06	22·07	22·76	22·36	22·24	20·65	17·80
55	18·12	18·00	18·49	15·79	15·74	15·95	16·45	16·48	15·85	14·76	12·49
Females.											
	H	H	F	E	E	E	B	S	L	M	
0	55·71	54·04	49·45	47·77	47·18	44·62	41·85	49·00	47·47	45·33	38·44
5	59·53	58·01	53·93	55·79	54·92	53·08	50·33	56·92	50·02	55·12	48·06
10	55·46	54·01	50·88	51·97	51·10	49·76	47·67	53·15	50·39	51·49	45·43
15	51·06	49·68	47·04	47·61	46·55	45·63	43·90	49·07	46·26	47·10	41·50
20	46·93	45·62	43·50	43·44	42·42	41·66	40·29	47·76	42·41	42·77	37·33
25	42·86	41·71	40·17	39·37	38·50	37·98	37·04	40·48	38·63	38·46	33·38
35	34·79	34·16	33·46	31·52	31·16	30·90	30·59	32·48	31·37	30·42	26·30
45	26·84	26·56	26·46	24·20	24·05	24·06	24·06	25·07	24·27	23·29	19·79
55	19·12	19·06	19·24	17·24	17·23	17·33	17·43	18·48	17·42	16·72	13·91

dividing the former by the corresponding sums from Table IV. The true death rate figures are then calculated by the formulæ given in the earlier part of the paper (Table III), and the expectation of life at each age obtained by dividing 1000 by each of the latter. It will be noticed that in Liverpool the true death rate is less than the corrected death rate, in other words that during the years referred to, Liverpool was using up life to a greater extent than she was creating it.

This includes all that need be said in the present communication, but a concluding table (Table VI) showing the expectation of life in all the life tables used above may not be without interest as few persons

possess access to all the data. These expectations are limited by the year 55. The ages above that come under a somewhat different law, and will be discussed in a subsequent paper.

The calculations in this article were made with the help of an Arithmometer supplied by the Carnegie Trustees.

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THE EFFECT OF CERTAIN DRUGS, TOXIC SUBSTANCES AND MICROORGANISMS ON THE FRAGILITY OF THE RED BLOOD CORPUSCLES OF MAN AND ANIMALS.

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(With 10 Charts.)

INTRODUCTION.

AMONG the properties of the red blood corpuscles, that have from time to time been investigated, the resistance of this type of cell to the destructive action of various lytic agents occupies a place of some importance. Very many substances have been studied from this point of view, and no useful purpose would be served by enumerating them here; but it should be noted in passing that, as regards the great majority of them, we have no evidence that they play any part in blood destruction as it occurs 'in vivo' under either physiological or pathological conditions.

Ever since the idea first occurred to Donders of applying the work of De Vries on the action of certain salt solutions on plant cells to animal cells and especially to red corpuscles (an idea which was followed up in the long series of investigations by his pupil, Hamburger, and by von Limbeck) the phenomenon of the laking of erythrocytes by hypotonic salt solutions has occupied the attention of the greater number of the workers in this field of research. It is to the study of certain aspects of this type of cell destruction that this investigation has been devoted.

¹ The earlier part of these investigations was carried out at St Thomas's Hospital while holding the Salters' Research Fellowship.

It will be more convenient to reserve all references to the work of the numerous investigators who have added to our knowledge of this subject for those portions of the paper in which the results and conclusions of each can be directly correlated with the experimental data obtained during the course of this research.

It is however desirable to enquire, at the outset, what significance may be attached to any alterations noted in the resistance of the erythrocytes to lysis of this type. The original conception put forward by Hamburger was that the red cell consists of an envelope having many of the properties of an impermeable membrane, containing within it certain salts and complex organic substances, among them haemoglobin, the whole suspension or solution exerting a definite osmotic pressure. Without the corpuscle is the serum, again consisting of a complex of salts and organic compounds, and which also exerts its particular osmotic tension, this tension being in excess of the tonicity of the corpuscular contents as measured against saline solutions. These comparatively simple physical conditions are implicitly assumed by Hamburger as the basis on which he explains his experimental results, and acting upon it, he was able to deduce that the isotonic value of a salt varied with its molecular weight, in which respect his work was soon after confirmed by von Limbeck. Von Limbeck, however, was far from believing that Hamburger's hypothesis sufficed to explain the phenomena observed. He notes that corpuscles are gradually lysed when allowed to remain in their native serum outside the body, and that therefore some chemical change must take place: secondly, that within the corpuscle there exists a fluid with much albumen and little salt, while in the serum there is usually less and quite different albumen, and more salt; so that either the albumen must affect the osmotic pressure or else part of the chlorides in the serum cannot enter into the osmotic action. He finds that, in the case of the blood of the horse, the isotonic value of the corpuscles measured against sodium chloride solution is 0.56%, whereas the serum contains only 0.46% of this salt, and therefore some other factor must be present to produce the isotonic relations. In human blood corpuscles, he found that the amount of sodium chloride was 0.2%, their isotonic equivalent 0.4% while the salt content of the serum was 0.62%, again clearly proving the interposition of other factors. More recently, we have the extremely interesting work of Kiss, who endeavoured to demonstrate the intimate relation between the haemolytic action of the solutions of various salts and the position of their constituent atoms in the periodic system of the elements. He proves,

moreover, in the course of this investigation, that a lowering of the temperature results in an increased haemolysis in any given concentration of saline. This fact was independently observed by Lewis, and is, of course, exactly the reverse of that which would obtain were Hamburger's hypothesis correct. It may be taken, then, that the conception that haemolysis in hypotonic saline solutions results from the rupture of a hypothetical cell membrane, produced by changes in the salt content and hence in the osmotic pressure within and without it, is definitely disproved.

We must now turn to the deeply interesting studies of Nolf. This investigator shows that all chemical substances, whose solutions produce haemolysis, may be divided into two classes. The members of one of these, such, for example, as weak solutions of urea, act as does distilled water. It suffices to add to them the amount of sodium chloride necessary to raise the tonicity of the solution to the usual limit to prevent haemolysis. The other class, and chief among its members is ammonium chloride, acts on the red cells and produces haemolysis even in the presence of sufficient sodium chloride to raise the tonicity of the solution to the normal value. Now, Nolf's conception is briefly this. He considers that there are three factors, the plasma within the cell with its salts and haemoglobin, the cell stroma and the serum, all of which possess a certain avidity for water, due both to their salt content and to other causes. Now, progressive dilution will increase the amount of water taken up by each of these three systems, and he believes that there is a definite critical point, beyond which absorption of water by the stroma produces a change in the physical relations between this constituent of the cell and the plasma and haemoglobin, which allows the diffusion of the latter. He particularly combats any idea of a mechanical rupture of any envelope or membrane. He considers that those chemical agents which belong to the same class as weak urea solutions act in the same way as distilled water; when their addition has brought about a certain dilution of the ambient fluid, and hence a certain definite "hydration" of the stroma, the critical point is reached and haemoglobin diffuses. Substances such as ammonium chloride, however, act in an entirely different manner, they specifically increase the power of the stroma to absorb water, so that a less dilution of the ambient fluid becomes necessary to produce the critical "hydration" of this element. Now, Nolf considers that all specific haemolysins, including specific haemolytic sera, act in this same manner. It is not possible to detail the experimental evidence that he adduces, but, briefly, he proves

that a haemolytic serum has no peptonising action on the red cells, and that increasing the tonicity of the saline solution in which the erythrocytes are suspended inhibits the action of both ammonium chloride and of a specific haemolysin; according to his hypothesis, by balancing the increased avidity of the stroma for water by the increased osmotic tension of the ambient fluid. However that may be, his observations on the inhibitory effect of increased saline concentrations on the action of specific haemolysins have been amply confirmed by Sutherland and McCay and also in the course of the present research. It is also of interest to note that ammonium chloride, alone of the chemical substances studied, ceases to exert its specific action at 0° C.

We must note that the validity of Nolf's hypothesis, or of some similar conception, is in no way affected by the observation of Peyton Rous, that alterations in the fragility of the red cells to hypotonic saline lysis on the one hand, and to haemolysis by a specific serum on the other, bear no definite relation to one another in the pathological conditions studied by him. Nolf does not state that haemolysis by a specific haemolysin and by hypotonic saline, or distilled water, is one and the same phenomenon; but that the action of the haemolysin produces a condition of the cell which enables a certain change to occur in a solution of high tonicity, which would in any event occur in a solution of low tonicity. It is, therefore, well within the bounds of possibility that in some cases, a change in the stroma which would render it more fragile, as regards the action of weak saline solution, would render it less liable to the action of a specific haemolysin. At all events, any change in the composition of the stroma might very well affect both factors, and there is not the least reason why it should affect both in the same way.

That hypotonic saline lysis is a change that has any place in the physiological or pathological processes of the body is in the highest degree improbable: that the effect of the tonicity of the body fluids on the action of haemolytic agents of other types may be a factor of considerable importance, is, on the other hand, an undoubted possibility.

In studying alterations in the fragility of the erythrocytes to weak saline solutions, we are dealing with a phenomenon which occurs, well-marked, in certain definite states of disease, and hence a condition concerning which any further information is of definite value.

In the present investigation, an effort has been made to obtain information on the following points,

1. The effect of the administration of certain arsenical compounds on the red cell fragility.
2. The effect of bile and of certain of its constituents.
3. The effect of certain pathogenic micro-organisms, known to be associated with haemolytic phenomena.
4. The effect of specific haemolytic sera.

Whenever, in the following pages the term 'fragility' occurs unqualified it is intended to denote fragility to hypotonic saline solutions. The term 'resistance' is used in an exactly inverse sense.

Technique.

The methods employed by different workers have varied in every possible direction; in the method of preparation of the saline solutions, in the use of washed or unwashed blood corpuscles, in the ratio between the volume of blood or red cells and the volume of saline solution to which they are added, and in the method of estimating the degree of haemolysis produced.

It was realised that the present research would involve a large number of readings, taken often at very short intervals, as, for instance, following the intravenous inoculation of a highly toxic substance; and hence it was essential to employ some method which would allow reasonably rapid readings and would not involve long and laborious examinations during each estimation. The method adopted, therefore, was the determination of the point of commencing haemolysis, *i.e.* the exact strength of the saline solution in which a faint trace of haemolysis occurred, the solution next higher in the series remaining entirely untinged. This was combined with such information regarding the degree of haemolysis in the lower concentration of saline as could be obtained from observation of the series of tubes put up in each case. The investigation, therefore, resolved itself in the main into the determination of the fragility of the cells of minimal resistance. The maximum resistance of the red cells would be determined by noting the strength of saline in which complete haemolysis occurred, but this point is very much harder to determine than the point of commencing haemolysis. There is a marked retardation in the relative number of cells destroyed in successive tubes as the saline concentration increases, so that several tubes intervene between that in which almost all the cells are destroyed and that in which every cell is lysed, and as the tints produced are indistinguishable, the determination has to be made on the presence of

a microscopic deposit after centrifugalisation or the detection of a very faint opalescence on shaking. The appearance of the first faint tinge of haemolysis in the series of tubes is however much more easily and certainly determined. Wherever possible the determinations were made in bright daylight, since this illumination is much the most satisfactory. In those cases in which artificial light had to be employed it was found that the use of a background possessing a slight greyish tint gave better results than one of a dead white colour.

It is not suggested that this method is so satisfactory as that followed for instance by Smith and Brown, in which the degree of haemolysis in each of a long series of tubes was accurately noted, using standard solutions of haemoglobin and suspensions of cells prepared from the actual corpuscles under examination, and the results plotted in curves giving the amount of haemolysis at each dilution. But it is changes at the lower end of the scale of resistance that have been the subject of most careful study; and it is almost certain that it is such changes which are the significant factors in actual pathological processes, if the fragility to hypotonic saline lysis really indicates any increased tendency to blood destruction in the body; since those cells of minimal resistance will obviously offer the first point of attack. The actual technique employed varied little from that adopted by Butler in his recently published observations on the fragility of the red blood cells in various pathological conditions, and this in its turn was a modification of the method employed by Dudgeon. The exact details of these methods will be found in the original papers. The details of the technique employed in the course of the present investigation are as follows:—

Preparation of saline solutions.

The basis of all the standard solutions was an accurate 1% solution of pure sodium chloride (Kahlbaum) in freshly distilled water, which was renewed at frequent intervals. The purity of the salt is obviously a matter of the first importance, and the routine use of Kahlbaum's preparation was found to be the only satisfactory method of ensuring this. Several samples supplied from other sources as "Pure Sodium Chloride," and employed in a few of the earlier experiments gave discordant results. The 1% saline solution was then placed in a 50 c.c. burette and a similar burette was filled with freshly distilled water. Two covered glass vessels were then taken and into one was run, say 5 c.c. of the 1% saline and 5 c.c. of distilled water, into the other 7 c.c.

of saline and 3 c.c. of distilled water, thus giving a 0.5% and 0.7% saline solution respectively. A series of small glass tubes of 2 c.c. capacity were then placed in a rack and into the first was placed 10 volumes of the 0.5% saline, into the second 9 volumes of 0.5% saline and 1 of 0.7% saline, into the third 8 volumes of 0.5% saline and 2 of 0.7% saline, and so on, the last tube containing 10 volumes of 0.7% saline. Thus, a series of solutions was prepared differing by 0.02% and ranging from 0.50%, 0.52%, 0.54% etc. to 0.70% saline. In Butler's method, solutions varying by 0.025% were prepared directly from the burettes and used in making up the dilutions of blood. The experience obtained during the course of these experiments has convinced me that a difference of about 0.02% approximates to the degree of sensitiveness of which this method is capable, and hence the method adopted has no advantage in point of accuracy over that employed by Butler, and it involves an additional stage of preparation. It possesses however one very real advantage. It was often necessary to be able to record a rapid change in fragility, the extent of which could not be foretold in advance, and hence a series of tubes, which would give results of the required accuracy immediately before an injection, would have to be extended to a cumbersome length if the possible variation was to fall within the limits of a second series, the individual solutions of which varied by a like amount. By varying this difference between successive tubes, this difficulty was obviated. Thus, using a 0.50% and a 0.60% solution a first series giving readings to 0.01% could be employed immediately before an injection. If previous experience suggested that a rapid alteration would probably follow, the second series could be prepared from a 0.50% and a 0.80% solution, giving differences of 0.03% between successive solutions, while, when the range of variation was determined, a series of tubes varying by 0.01% or 0.02% could be rapidly prepared, in which a range of eleven tubes would be certain to include the critical strength of solution and allow an ample margin on either side. This elasticity of arrangement gives a great advantage where several series of tubes of differing values are required during a short space of time. It is cumbersome and clumsy to prepare less than 10 c.c. of a saline solution accurate to two places of decimals per cent. from a burette, employing 1% saline and distilled water as the parent solutions, and it is useless to prepare 10 c.c. when very possibly only 1 c.c. of that particular strength will be needed. As regards the use of capillary pipettes with a single mark, using an air-bubble to measure off volumes, as against the employment

of graduated pipettes; the former method was at first adopted, but it was afterwards found that the use of 1 c.c. pipettes, graduated in tenths, added much to the rapidity and comfort of the manipulation where many tubes had to be put up.

Addition of blood or red cells.

The great majority of the estimations were made on blood, as such, and here $\frac{1}{20}$ th of the volume of saline to be used as diluent was employed. Dudgeon employed $\frac{1}{11}$ th of the total volume, Butler $\frac{1}{10}$ th and the method here adopted gives $\frac{1}{21}$ th. This measure was employed, since for any given total volume it necessitates a smaller quantity of blood, and this in the case of a moribund rabbit is a very great advantage. Thus, in the very great majority of these experiments, where 1 c.c. of saline was present in each tube, 0.05 c.c. of blood withdrawn in a fine graduated pipette, was added. Where washed corpuscles were employed, they were received into saline and citrate, deposited after an interval by centrifugalisation, washed again in saline and citrate, then once in saline, and centrifuged at high speed for several minutes, the thick deposit of cells from the last washing being employed for putting up the dilutions. In all cases sedimentation of the cells, so that the degree of tingeing of the supernatant fluid could be examined, was produced by centrifugalisation. As regards the time allowed to elapse between collecting and mixing the blood or red cells and centrifuging the mixtures; a series of preliminary experiments showed that, while slight changes occurred in the degree of haemolysis during the first 30 minutes, there was very little alteration after that time until two to three hours had elapsed, whereas, after long periods the degree of haemolysis in all tubes steadily increased. An uniform interval of 30 minutes was therefore allowed to elapse between preparing the mixtures and centrifuging them. The major part of this investigation has taken the form of observations on the fragility of the red blood cells of animals following inoculation with various toxic and other substances. The animals employed throughout have been rabbits. The marginal auricular vein of these animals affords an excellent site both for venipuncture for obtaining blood for examination, and for intravenous inoculation. In almost every case the marginal vein of one ear was employed for administering the injection, that of the other ear for withdrawing the blood. The blood was obtained by simple veni-

puncture. 0.05 c.c. was immediately drawn up into a calibrated pipette and expelled into one of the tubes of saline solution, and then mixed by drawing up and expelling the suspended red cells two or three times. In cases in which the cells were to be washed previous to examination the blood was allowed to flow straight into saline and citrate solution and was thereafter treated as stated above.

Red cell fragility in normal rabbits.

It may be noted that the normal fragility of rabbits' red cells was found to correspond to commencing haemolysis in 0.54 to 0.58% saline. Of 41 rabbits whose blood was examined during the course of this investigation, five only showed any marked variation from these figures. All these alterations were in the direction of abnormally low values for the saline corresponding to the first trace of lysis, which in two cases was 0.46%. In some of the experiments, in which low readings were obtained, a specimen of "Pure Sodium Chloride" was employed that was by no means above suspicion, but this would hardly account for such wide departures as the above. The figures obtained in the great majority of cases for commencing haemolysis, agree closely with those mentioned by most of the workers who have employed rabbits' red cells, von Limbeck—0.55% saline, Cornwall—0.58% saline¹,—etc.

On the other hand, French workers seem to have obtained consistently lower values, Nolf—0.46% saline and Foix and Salin—0.41% to 0.46% saline. In this connection it is of considerable interest to recall earlier experiments of Theobald Smith, in which the abnormal corpuscular fragility met with, in certain of the anti-toxin horses he examined, led him to believe that the repeated abstractions had produced this variation, while widely extended researches, carried out in conjunction with H. R. Brown, convinced him that he was in reality dealing with an individual peculiarity. A considerable series of preliminary experiments were carried out in order to determine whether the corpuscular fragility of the rabbit is a non-variable factor. Repeated examinations carried out over many weeks on any individual rabbit yielded remarkably constant results, variations greater than those corresponding to 0.02% saline being very unusual.

¹ This observer found that the average range of lysis for all the animals examined corresponded to 0.144% NaCl. He gives the mean lytic point for the rabbit as 0.51% NaCl.

Effect of injecting normal saline, etc.

Similar determinations were made on animals which were subjected to intravenous and intraperitoneal inoculations of normal saline, and on animals which had suffered a few blood abstractions of moderate amounts, but no variation was noted. In view of the importance which has recently been attributed to the absolute purity of the distilled water used for preparing saline solutions which are employed in animal inoculation, a certain number of experiments were made with saline prepared from distilled water which had stood in an iron tank for some eight months and was obviously grossly contaminated. It produced no variation whatever in the corpuscular fragility. The distilled water actually employed in the whole of the following series of experiments was freshly distilled from a glass vessel, using an ordinary glass condenser which was kept perfectly clean.

The effect of arsenic and of certain arsenical compounds on the fragility of the red cells of man and animals.

Gunn, as the result of certain 'in vitro' experiments, suggests that the administration of arsenic produces an increased resistance of the erythrocytes to hypotonic saline haemolysis, and believes that the beneficial effect produced by compounds of this element in Pernicious Anaemia may be explained on these lines. The results obtained by the various workers who have estimated the red cell fragility in this disease have been somewhat discordant, and Butler, who examined four cases without finding any appreciable variation from the normal in this respect, has suggested that the administration of arsenic which is so generally resorted to in these patients, may introduce a disturbing factor and mask the true state of affairs.

For this reason, and also because of the common employment of arsenical preparations in blood diseases, it appeared to be of some importance to gain further knowledge of the effect of the administration of drugs of this class on the corpuscular fragility.

1. Administration of arsenic (arsenious acid) to human beings.

The red cells of several patients, who were taking this drug in ordinary therapeutic doses as treatment for various conditions, were examined without finding the slightest departure from the normal in any case. In no instance had the drug been pushed far enough to produce any toxic effect.

2. *Administration of salvarsan (dioxylamidoursenobenzol) to human beings.*

The blood of several patients who received injections of this drug was examined before its administration and at intervals varying from ten minutes to six days afterwards. In the majority of cases the drug was inoculated intravenously, and in all of these only one injection of 0·6 gramme was given. In one case the drug was administered intramuscularly, and here repeated inoculations were given. The blood cells of this patient were examined on 14 different occasions during a period of one month. They showed throughout a somewhat high fragility, commencing haemolysis occurring with 0·42% to 0·47% saline, but this abnormality was present before the administration of the drug was commenced and showed no constant increase during the period of examination. There is, therefore, no evidence that the increased fragility was in any way connected with the mode of treatment.

The patient was suffering from a Syphilitic Transverse Myelitis. In every other case of this series the results obtained fell within normal limits.

3. *The inoculation of arsenic (arsenious acid) into rabbits.*

Experiment.

A small rabbit was selected and the fragility of its red cells determined on two separate occasions. A 1/400 solution of arsenious acid in sterile saline was prepared, just sufficient hydrochloric acid being added to produce complete solution. Varying amounts of this solution were injected intraperitoneally and the red cell fragility was determined at intervals. The results obtained are tabulated on p. 203.

The animal showed only slight wasting until 30. 1. 13 when it became obviously ill, and eventually died a few hours after the last injection. On 31. 1. 13 and at all subsequent examinations a slight degree of haemoglobinaemia was present, so that the colouration decreased not to a completely colourless fluid but to one faintly tinged with haemoglobin. Since, however, a long series of tubes was employed at each examination, it was not difficult to observe the first tube in which the added tinge, due to the hypotonic saline haemolysis, was present.

The result of this experiment is shown graphically in Chart 1.

EFFECT OF THE INOCULATION OF ARSENIUS ACID INTO A RABBIT

= STRENGTH OF SALINE SOLUTION CAUSING FIRST TRACE OF HEMOLYSIS.

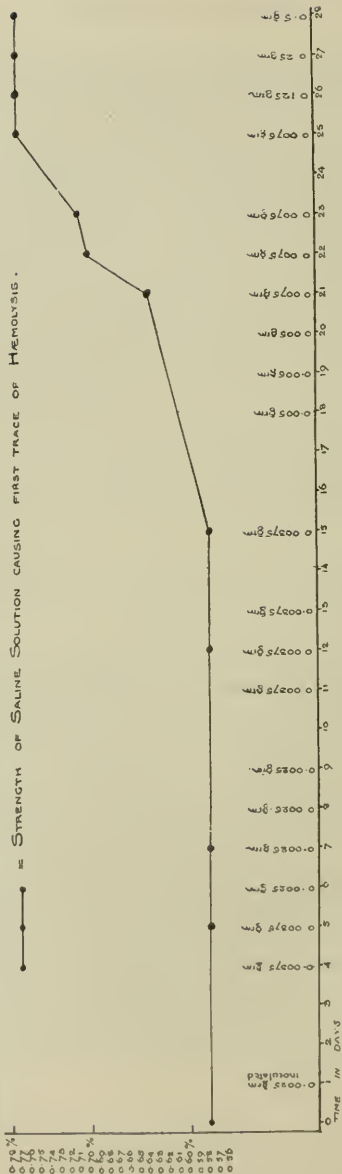


Chart I.

Date of examination	Amount injected	Strength of saline solution causing first trace of haemolysis
Before injection		0.58 %
10. 1. 13	0.0025 gm.	—
13. 1. 13	0.00375	—
14. 1. 13	0.00375	0.58
15. 1. 13	0.0025	—
16. 1. 13	0.0025	0.58
17. 1. 13	0.0025	—
18. 1. 13	0.0025	—
20. 1. 13	0.00375	—
21. 1. 13	0.00375	0.58
22. 1. 13	0.00375	—
24. 1. 13	0.00375	0.58
27. 1. 13	0.005	—
28. 1. 13	0.005	—
29. 1. 13	0.005	—
30. 1. 13	0.0075	0.64
31. 1. 13	0.0075	0.70
1. 2. 13	0.0075	0.71
3. 2. 13	0.0075	0.77
4. 2. 13	0.0125	0.77
5. 2. 13	0.025	0.77
6. 2. 13	0.5	0.77

Experiment.

A second rabbit received a single inoculation of 0.25 gramme of arsenious acid intraperitoneally. Its blood cells were examined before the inoculation and at intervals of 45 minutes and three hours afterwards, with the constant result that haemolysis commenced in 0.58% saline. Two hours later the animal developed acute toxic symptoms and died within 15 minutes, and, as is so often the case in moribund animals, only a drop of blood could be obtained from the veins of the ear. Immediately after death blood was obtained from the axillary vessels and this showed the first trace of haemolysis in 0.68% saline.

As a result of these experiments it will be seen that it is only when the arsenic is pushed to highly toxic doses that any effect is produced, and that this is in the direction of increased fragility.

4. The effect of atoxyl (meta-arsenic-anilide) on the fragility of rabbits' red cells.

A certain number of 'in vitro' experiments were first performed to test the action, if any, of atoxyl on the washed red cells; the action was tested in solutions varying from 1% to 0.001% of the drug in

normal saline. In some series of experiments the cells were washed after the drug had been allowed to act for 30 minutes at 37° C., in others the mixtures were centrifugalised at high speed after incubation, the supernatant fluid pipetted off and the red cells immediately added to the saline solution. The results are quite without interest and need not be elaborated here.

Experiment.

A rabbit received an injection of 0·2 gramme of atoxyl into the lateral auricular vein. The red cells were examined before the injection, 5 minutes, 1 hour, 3 hours, 24 hours and 48 hours afterwards without showing any alteration worthy of note. On the day following the inoculation the rabbit showed slight toxic symptoms, but these passed off completely in three days. On the seventh day after the first injection a second, consisting of 0·3 gramme of atoxyl, was given. The blood was examined immediately before the inoculation, 5 minutes, 15 minutes, 1 hour and 3 hours afterwards without showing any alteration. The effect on the rabbit however was almost instantaneous and of a most marked character. It became obviously ill within 15 minutes of the inoculation, respiration becoming extremely rapid and the animal being unable to stand, death in fact appeared imminent. Severe gastro-intestinal symptoms appeared within 12 hours and on the second day the hind limbs were completely paralysed. All symptoms except the paralysis passed off in seven days, during which time however the animal wasted considerably. In connection with these toxic symptoms a marked change occurred in the fragility of the red cells.

The percentage of sodium chloride in the tube which showed commencing haemolysis at the beginning of the experiment was 0·57. 24 hours after the second injection haemolysis occurred in 0·63 % saline. This value fell daily during the following week and on the seventh day was again 0·57 %. 10 days after this injection 0·5 gramme of atoxyl was administered. As previously the blood cells were examined before the inoculation and at varying intervals afterwards. No alteration in fragility occurred and the rabbit displayed no toxic symptoms of any kind. The blood was examined on several subsequent occasions but showed no change in the red cell fragility, and the rabbit recovered completely. The results of this section would appear to show that arsenical compounds administered in therapeutic doses have no action on the fragility of normal red corpuscles.

The effect of bile and of certain of its constituents on the fragility of the red blood cells of the rabbit.

Among all the pathological conditions affecting the human subject which have been studied from the point of view of alterations in red cell fragility, none have given more interesting and constant results than those associated with Jaundice. Von Limbeck first drew attention to the decreased corpuscular fragility in Obstructive Jaundice, and his results have been confirmed by Chauffard, Chalier, Vaquez and Ribierre, Peyton Rous, McNeil, Butler and others. This phenomenon seems to be closely associated with the Jaundice itself, since, in Butler's cases for example, the actual causative conditions included Gall-stones, Carcinoma of the pancreas, Pancreatitis, Secondary Carcinoma of the liver and Cirrhosis. It is also noteworthy that a decreased fragility has been recorded in cases of Septic Jaundice.

If now we consider another, much less common, condition associated with a certain degree of Icterus, namely Congenital Family Cholaemia, we are met with a wide variation in the opposite direction, *i.e.* with a markedly increased susceptibility to hypotonic saline haemolysis.

This interesting phenomenon, first noted by Chauffard, has been frequently confirmed by Peyton Rous, Tileston and Griffin, Chalier, Le Gendre and Brulé, Cade, Hawkins and Dudgeon, Hutchison and Pantou and Butler.

It becomes, therefore, a matter of interest and importance to try and determine what are the factors involved.

Von Limbeck originally offered the tentative suggestion that in Obstructive Jaundice the bile-acids might cause the haemolysis of the more fragile cells, leaving those of greater resistance. He abandoned this idea, however, on two grounds: (1) that it assumes that the tonicity of the serum sinks below the normal isotonic limits when bile-acids are present, while he himself found the opposite to be the case, (2) that it would involve an Oligocythaemia, which does not occur.

McNeil, in his communication on Saponin Haemolysis, suggests that the cause of the decreased fragility is a result of the hypertonicity of the serum. His reasoning is difficult to follow, and the results of his experiments on this point are in direct contradiction to those of Butler, with whose observations my own are in entire agreement. It is also, I think, the common experience of all, who work with red blood cells, that their tendency to haemolysis steadily increases when they are kept in saline solution after removal of the plasma by washing.

As regards the heightened tendency to saline haemolysis in Congenital Cholaemia, there is general agreement in attributing it to an inherent abnormality of the erythrocytes, evidenced in other ways by their altered shape, size and staining reaction.

The methods adopted during the course of this part of the investigation were as follows:

- (1) The inoculation of rabbits' bile into rabbits.
- (2) The inoculation of sheeps' bile into rabbits.
- (3) The inoculation into rabbits of bile-salt (sodium taurocholate), together with certain 'in vitro' experiments with this substance.
- (4) The inoculation of cholesterin into rabbits.

(1) *The inoculation of rabbits' bile into rabbits.*

The bile employed was collected from the gall-bladder immediately after death. It was heated at 58° C. for thirty minutes to ensure sterility, and this sterility was proved by culture before injection. Two experiments were made.

A rabbit received 2.5 c.c. of a $\frac{1}{50}$ dilution of rabbits' bile in sterile saline intravenously, and a second 2.5 c.c. of the same dilution 24 hours later. Repeated examinations of the red cells revealed no alteration in their fragility.

A second rabbit received 3 c.c. of a $\frac{1}{4}$ dilution of rabbits' bile intravenously. The red cells were examined at various intervals ranging from 5 minutes to 7 days after the inoculation, but no change in fragility occurred. Marked haemoglobinaemia was present 5 minutes after the injection but this gradually decreased and entirely disappeared in 24 hours.

(2) *The inoculation of sheeps' bile into rabbits.*

A rabbit received, on four successive days, injections of 2 c.c., 2 c.c., 3 c.c. and 2 c.c. of undiluted, sterile sheeps' bile intraperitoneally. Severe toxic symptoms supervened a few minutes after each injection but rapidly passed off. Repeated determinations of the red cell fragility showed no alteration. The bile in this case was not heated prior to inoculation.

(3) *The inoculation into rabbits of bile salt (sodium taurocholate) together with certain 'in vitro' experiments with this substance.*

Twelve rabbits were subjected to single or repeated injections of this salt, in doses which varied from amounts which caused no symptom of any kind to those which caused almost instantaneous death. The injections were given both intravenously and intraperitoneally.

No alteration of importance was noted in any case. In those cases where the doses given were so large as to cause rapid death, blood obtained post-mortem showed a moderately increased fragility, but the intrusion of this factor deprives these results of any value.

No useful purpose would be served by giving details of this series of experiments which involved a very large number of determinations. One experiment, however, may be briefly noted. A rabbit received repeated inoculations of sodium taurocholate over a period of 38 days. During this time 23 inoculations of this salt were given, commencing with 1 c.c. of a 0.5% solution administered intravenously, and terminating with 3 c.c. of a 10% solution administered intraperitoneally. The animal showed practically no toxic symptoms throughout the whole experiment and repeated estimations of its red cell fragility gave constant results.

A considerable number of 'in vitro' experiments were performed, consisting in the subjection of washed red cells, both human and rabbits', to the action of varying amounts of sodium taurocholate for different periods of time, both at 37° C. and at room temperature. The results were uniformly negative, so far as any change in fragility was concerned.

(4) *The inoculation of cholesterin into rabbits.*

McNeil has given reasons for believing that the lessened resistance of the red cells in Jaundice to Saponin Haemolysis is dependent on the presence in them of an abnormally large amount of cholesterin; and Thiele and Embleton have noted, in a recent study on the factors involved in Wassermann's Reaction, that cholesterin in some way alters red cells which have been in contact with it, so that more amboceptor-complement compound is required to haemolyse them, even after they have been centrifugalised and washed. It was thought, therefore, that it might be this constituent of bile which was especially concerned in the altered resistance to hypotonic saline haemolysis noted in jaundiced conditions.

The cholesterolin was injected intravenously as a fine suspension in sterile saline. Three out of four injections made in this way produced no ill effects, the fourth caused death within an hour. The toxic effect in this case was probably due to the physical condition of the suspension since no more cholesterolin was administered in this case than in the other three.

Intraperitoneal injections were given as fine suspensions in saline and as solutions in olive oil, and a solution prepared in this way was also inoculated intramuscularly. The amount of cholesterolin administered at one injection varied from 0.05 to 0.2 grammes. One rabbit received two intravenous injections, another three intraperitoneal and one intramuscular injection. In no case was any change in red cell fragility observed.

Thus, in the whole series of experiments made with the bile and its constituents the results were almost entirely negative.

*Alterations in the fragility of the red blood cells of rabbits
following the inoculation of certain micro-organisms.*

A considerable amount of data has been collected regarding the influence of acute bacterial infections on the fragility of the red blood corpuscles of man.

Von Limbeck records an increased fragility in Pneumonia, Erysipelas and Typhoid Fever, and quotes Cavazzani as obtaining a similar result in Typhoid after the fifteenth day of illness.

Dudgeon, in one case of Erysipelas, found an increased fragility comparable to that met with in cases of Congenital Cholaemia.

Butler examined six cases of acute bacterial infection, including three of Septicaemia, two of which proved to be due to a *Streptococcus*, one Streptococcal Arthritis, one streptococcal infection of a Hernia wound, and one case of Pyaemia. Four out of the six cases showed a slightly decreased fragility. One case of Erysipelas and one of Cellulitis showed a normal degree of fragility, while nine cases of Pneumonia showed a uniform slight decrease. In acute Rheumatism, this observer found the fragility to fall within normal limits.

G. B. Bianchi-Mariotti injected filtered cultures of various micro-organisms into rabbits and subsequently tested the fragility of their red cells with the following results:—filtered cultures of *B. anthracis*, *B. pyocyaneus*, *Streptococcus pyogenes* and *V. cholerae*, when injected in small or moderate doses, increase the fragility of the red cells, though often only to a slight degree. Moderate doses of a filtered culture of

B. typhosus produced a decreased fragility in 42 hours. Injections of large amounts of filtrate constantly caused a fall in the fragility. The author defines "moderate" doses of the filtrate as being, according to the nature of the organism and the age of the culture, from 3 to 6 c.cm. per kilo of the animal's body-weight. No full details of the experiments are given nor any numerical results stated.

Thus the evidence as regards the effect of micro-organisms and their products on the fragility of the red blood cells of man and of animals is of a peculiarly conflicting nature.

In the experiments to be described, attention was particularly directed towards those bacteria whose toxic products usually possess a certain haemolytic power; and in all cases living organisms were employed for inoculation.

1. *Inoculation of cultures of Streptococci.*

Experiment.

The fragility of the red cells of a small black rabbit was examined and found to be such that haemolysis commenced in 0.56% saline. 3 c.c. of a saline emulsion of a *Streptococcus* of the *pyogenes* type were injected into the muscles of the left thigh. The red cells were examined at intervals of 2½, 5¾, 7¾, 26, 29, 50 and 74 hours after the injection; but no deparature from the normal fragility was observed. The rabbit became temporarily ill, and some swelling of the thigh occurred, but this rapidly passed off, and complete recovery took place.

Experiment.

A small brown and white rabbit was selected and the fragility of its red cells determined. 1.2 c.c. of a saline emulsion of a 24 hours' agar culture of a *Streptococcus*, known to produce haemolysis, was injected intravenously. The red cells were examined at the following intervals and with the results shown.

Time of examination	Strength of saline solution causing first trace of haemolysis
Before inoculation	0.55 %
2½ hours after inoculation	0.55
6 " " "	0.56
7¾ " " "	0.54
26 " " "	0.61
29 " " "	0.59
50 " " "	0.55
74 " " "	0.56

The animal became obviously ill about 24 hours after the inoculation but gradually recovered.

Experiment.

A large rabbit was selected and the usual preliminary investigation was made. 3 c.c. of a 48 hours' broth culture of an actively haemolytic *Streptococcus* were injected intravenously. The red cells were then examined at various intervals. At each of these examinations a tube of blood was collected, allowed to stand for 30 minutes at 37° C., and then centrifuged and the supernatant serum examined for any tingeing with haemoglobin. This precaution was taken because this *Streptococcus* had produced haemoglobinaemia in experiments performed by Dr Walter Macleod, to whose kindness I am indebted for the cultures used in this and in the preceding experiment. It will be seen that after eight hours, the serum became faintly tinged with haemoglobin; so that it is clear that in this case one was not really working to a completely untinged solution; but it will be remembered that the serum is diluted at least $\frac{1}{40}$ th, and the faintly-tinged serum diluted to this extent gave no colour observable by the eye or by the spectro-scope, and the demarcation, between the tube in which the additional tinge caused by saline haemolysis was present and that in which it was absent, yielded no difficulty. The following were the results obtained:

Time of examination	Strength of saline solution causing first trace of haemolysis	Serum
Before injection	0.55 $\frac{1}{10}$	Nil.
1 hour after injection	0.67	Nil.
2 hours " "	0.67	Nil.
8 " " "	0.73	Faint tinge.
22 " " "	0.73	Faint tinge.
51 " " "	0.73	Very faint tinge.
55 " " "	0.70	Nil.
13 days " "	0.58	Nil.

The animal became acutely ill within two hours after injection, and at 24 hours it appeared moribund, but afterwards it gradually recovered, and at the end of a week appeared perfectly normal. The results of the earlier part of the experiment are shown in Chart 2.

Experiment.

A rabbit was selected and a preliminary determination made of the fragility of its red cells. The first trace of haemolysis was found to occur in 0.54% saline. One c.c. of a saline emulsion of a 24 hours' agar culture of the *Streptococcus pyogenes* was injected intravenously. The fragility of the red cells was examined after 3, 6, 26, 29, 54

and 74 hours, the result in each case being identical with that obtained before the injection. The animal showed a marked indisposition, lasting from 12 to 24 hours, and then completely recovered. The *Streptococcus* employed in this case was isolated from an infected hernia wound.

EFFECT OF THE INTRAVENOUS INJECTION OF 3 C.C. OF A 48 HRS.
BROTH CULTURE OF A VIRULENT *STREPTOCOCCUS*.

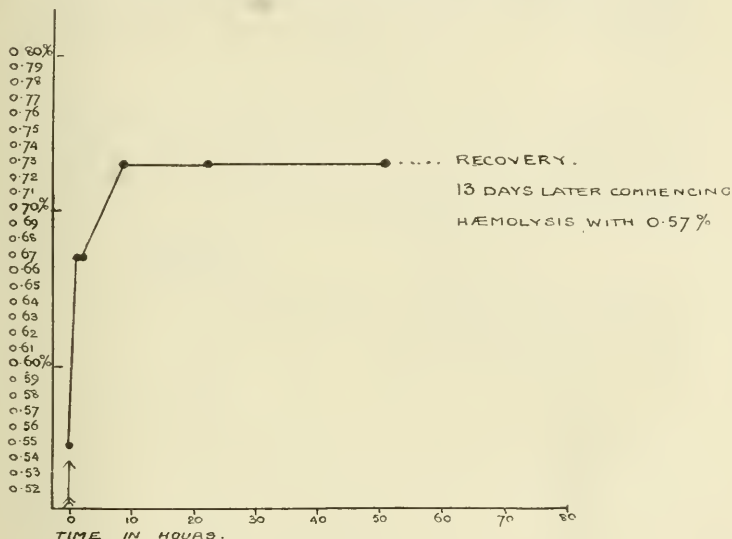


Chart 2.

Thus, out of four rabbits inoculated with different strains of *Streptococci*, two showed no alteration in their red cell fragility, one showed a slight rise, and one a very considerable increase persisting for several days. It is noteworthy that this occurred in the rabbit inoculated with a 48 hours' broth culture, which thus contained in addition to the organism itself the haemolysin which Macleod has shown to be present in filtered cultures. Another noteworthy circumstance is the ultimate recovery of all four animals, and also the fact that the rabbit exhibiting the most marked rise in red cell fragility became the most acutely ill.

2. Inoculation of cultures of the *Staphylococcus aureus*.*Experiment.*

A small white rabbit was injected intravenously with 1 c.c. of a saline suspension of a 24 hours' culture of the *Staphylococcus aureus*. Before inoculation the first trace of haemolysis was observed in 0.46% saline. Half-an-hour later the tube containing 0.47% saline showed distinct tingeing. The rabbit, unfortunately, became acutely ill and died within a few hours, before another estimation was made. The very slight rise here observed renders the result of little value.

Experiment.

A small brown rabbit was selected and a preliminary determination of its red cell fragility made. Various inoculations of saline suspensions of the *Staphylococcus aureus* were performed, an agar culture 24 to 48 hours' old being used in each case in the preparation of the suspension. The results obtained were as follows:—

Time of examination	Strength of saline solution causing first trace of haemolysis
Before inoculation	0.49 %
Inoculation of 2 c.c. of saline suspension of the <i>Staphylococcus aureus</i> , intravenously.	
6 hours after inoculation	0.51
26 " " "	0.51
30 " " "	0.51
48 " " "	0.51
54 " " "	0.55
76 " " "	0.58
Inoculation of 2 c.c. of saline suspension of the <i>Staphylococcus aureus</i> , intravenously.	
90 hours after first inoculation	0.57
100 " " "	0.57
120 " " "	0.62
Inoculation of 2.5 c.c. of saline suspension of the <i>Staphylococcus aureus</i> , subcutaneously.	
124 hours after first inoculation	0.63
144 " " "	0.61
216 " " "	0.60
240 " " "	0.61
Inoculation of 0.5 c.c. of saline suspension of the <i>Staphylococcus aureus</i> , intravenously, and 2 c.c. intraperitoneally.	
264 hours after first inoculation	0.63
268 " " "	0.63
282 " " "	0.60
286 " " "	0.60
310 " " "	0.58
331 " " "	0.58
358 " " "	0.57

Two subcutaneous abscesses developed towards the end of the experiment and the rabbit wasted rapidly; it was therefore killed on the 16th day.

The results of the earlier part of this experiment are shown in Chart 3.

3. *Inoculation of cultures of the Bacillus pyocyaneus.*

Experiment.

A small brown rabbit was selected and after the fragility of its red cells had been determined, 1 c.c. of a saline emulsion of a stock laboratory culture of the *B. pyocyaneus* was injected intravenously. The results were as follows:—

Time of examination	Strength of saline solution causing first trace of haemolysis
Before inoculation	0.46 %
$\frac{1}{2}$ hour after inoculation	0.46
1 hour " "	0.47
19 hours " "	0.50

When the last estimation was made the animal was 'in extremis,' and, as is so often the case under these conditions, it was impossible to obtain more than a drop of blood from the veins of the ear. The rabbit was killed and the subclavian artery immediately cut across, the blood taken from this source being employed in the tests. This detracts somewhat from the value of the results.

Experiment.

A small brown and white rabbit was selected and after the usual determination had been made, 1.5 c.c. of saline emulsion of a stock laboratory culture of *B. pyocyaneus* were injected intravenously. The results obtained were as follows:—

Time of examination	Strength of saline solution causing first trace of haemolysis
Before inoculation	0.50 %
$2\frac{1}{2}$ hours after inoculation	0.53
6 " " "	0.53
26 " " "	0.57

Death occurred within a few hours of the last estimation. The results of this experiment are shown graphically in Chart 4.

EFFECT OF THE INTRAVENOUS INJECTION OF 1·5 C.C.
OF A SALINE SUSPENSION OF *B. PYOCYANEUS*.

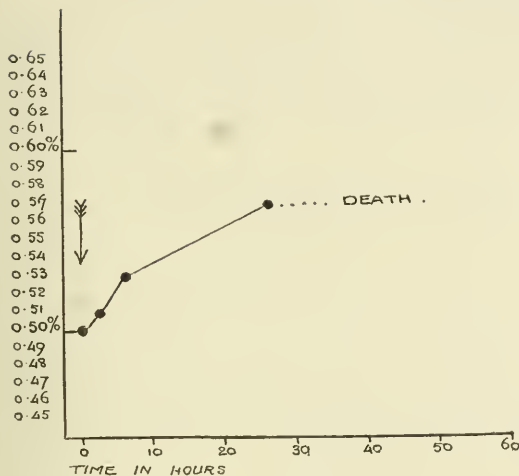


Chart 4.

4. *Inoculation of cultures of the Bacillus of Danysz.*

Only one experiment was performed with this organism.

Experiment.

Small brown rabbit.

Intravenous injection of 2 c.c. of a saline suspension of a young agar culture of the bacillus of Danysz.

Time of examination	Strength of saline solution causing first trace of haemolysis
Before inoculation	0.58 ⁹ / ₁₀
3 hours after inoculation	0.62
6 " " "	0.64
26½ " " "	0.62

Death occurred within a few hours of the last examination.

The results of this experiment are shown graphically in Chart 5. Thus, we may summarise the results of this part of the investigation by saying, that of certain of the bacteria, known to cause haemolysis under various conditions, almost all the strains examined produced, on inoculation, an increasing fragility, usually slight, but sometimes well marked.

The effect of haemolytic sera on corpuscular fragility.

The relation between hypotonic saline haemolysis and haemolysis due to specific haemolysins has been already considered, and the important work of Nolf, Peyton Rous, Kiss, Sutherland and McCay and others briefly referred to. We must now consider, in greater detail, the actual effect produced on the red blood cells by haemolytic sera 'in vivo' and 'in vitro,' and it will be more convenient to commence with the former.

EFFECT OF THE INTRAVENOUS INJECTION OF 2 C.C.
OF A SALINE SUSPENSION OF *E. DANTZS*.

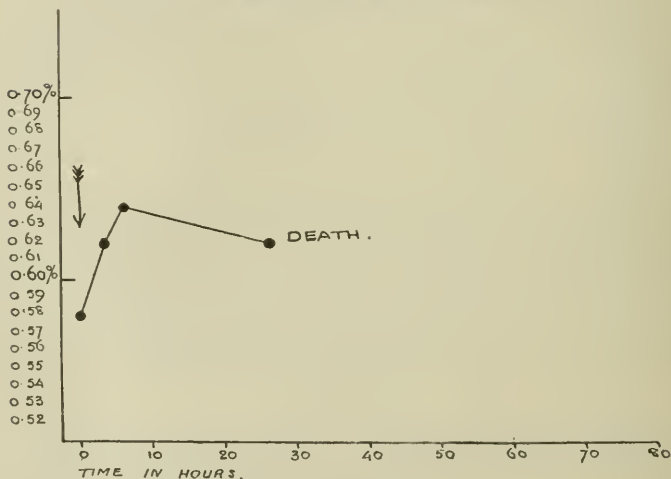


Chart 5.

1. *The inoculation of specific haemolytic sera into rabbits.*

We already possess definite evidence that the injection into animals of specific haemolysins results in an increased fragility of the red blood cells. Christophers and Bentley, in the course of their valuable research on Blackwater Fever, injected into dogs considerable quantities (15 c.c.) of the serum of goats, which had been immunised against dogs' red cells, and obtained variations in fragility corresponding sometimes to 0.3% or

even 0.4% NaCl. They also inoculated Daboia Venom into rabbits and obtained similar results, while the specimen of Cobra Venom employed by them was much less active. These workers, however, in this part of their research, employed solutions showing wide differences of concentration from tube to tube (0.05% to 0.10%), and they consider such alterations as that indicated by the occurrence of commencing haemolysis in 0.45%, 0.52%, and 0.40% saline in successive determinations made during the course of a few hours as unimportant. Certain points brought out by them are of great interest. They note that increased fragility occurs in the complete absence of either haemoglobinaemia or haemoglobinuria, and they also point out that the resistance of the red cells regains its normal limit after several days in those animals which survive, and that at this time large cells with polychromatophilic staining are present. More recently Foix and Salin, in a paper dealing with the experimental study of Paroxysmal Haemoglobinuria, have found that a constant increase in fragility followed the injection into rabbits of fresh and heated human serum, though this rise is seldom considerable in degree. The actual values they obtained differ very considerably from my own, since they give a normal maximum resistance corresponding to 0.41% to 0.46% saline for rabbits' red cells, figures which differ widely from those obtained by the majority of workers; on the other hand, they correspond closely with those mentioned by Nolf. In view of the importance of this question, it seemed desirable to study these phenomena in more detail by the methods described above.

The specific haemolytic sera employed were, in all cases, prepared by repeated intraperitoneal injections of the washed red cells of rabbits into guinea-pigs. In some cases the serum was heated before use, in others it was employed in the fresh state.

Experiment.

A serum haemolytic for rabbits' red cells was prepared in the above manner, and labelled serum 'α.'

No determination of its precise strength, when acting 'in vitro,' was made, and the serum was not heated to destroy complement. A normal rabbit was selected and the fragility of its red blood corpuscles determined. One c.c. of haemolytic serum 'α' was then injected intravenously. The following were the results obtained:—

Fragility of Erythrocytes

Time of examination	Strength of saline solution causing first trace of haemolysis
Before injection	0.54 %
3 hours after injection	0.60
6 " " "	0.63
26 " " "	0.69
30 " " "	0.70
54 " " "	0.69
74 " " "	0.66
96 " " "	0.67
108 " " "	0.62

The animal showed no clinical manifestation of ill-health during the whole course of the experiment. Thus, the injection of 1 c.c. of this serum intravenously was followed by a rise in fragility corresponding to 0.14% saline in 6 hours, and to 0.16% saline in 30 hours.

Experiment.

A second rabbit received 0.25 c.c. of the same serum intravenously. The results were as follows:—

Time of examination	Strength of saline solution causing first trace of haemolysis
Before injection	0.54 %
4 hours after injection	0.57
24 " " "	0.63
48 " " "	0.60

As in the last experiment, the animal remained to all appearances perfectly well.

Thus, comparatively small doses of a haemolytic serum, judged from the clinical effects, produced in rabbits a marked rise in corpuscular fragility.

It was most natural to suppose that these changes were produced by the actual action of the haemolysins on the red cells circulating in the blood, but certain other possibilities were present which had to be eliminated.

Muir and McNee, in an extremely interesting paper on the Anaemia produced by a haemolytic serum, describe the effect caused by inoculating rabbits intravenously with a goat *v.* rabbit immune serum. They found that an enormous reduction occurred in the number of red cells in the peripheral circulation, commencing immediately but only reaching its maximum about the third day in the majority of cases. This phenomenon was usually accompanied by such evidences of blood destruction as haemoglobinaemia and haemoglobinuria, although in one case it is

specifically mentioned that although haemoglobinuria was found to be present on two examinations, within a few hours of the injection of the serum, the rabbit's own serum showed no trace of tingeing. The point, however, which is of especial moment in connection with the present research, is that the blood destruction, in Muir's and McNee's experiments, was in all cases associated with the appearance in the peripheral circulation of various types of abnormal red cells, basophilic cells and nucleated red cells of all types, often in enormous numbers. They usually appeared late, about the third day, but sometimes much earlier, and then rapidly increased in numbers. It obviously becomes a question of moment whether the increased fragility, observed in the corpuscles of the peripheral blood, in such cases, is in reality due to the entrance into the circulation of abnormal cells possessing an abnormal fragility. To determine this point, a series of experiments were instituted, in which simultaneous determinations were made of the fragility of the red cells, the number of corpuscles per cmm. of peripheral blood, the morphological characteristics of the cells present and the presence of any tingeing of the serum. The haemolytic serum employed in this series of experiments was prepared in the same way as serum 'α.' It was employed unheated and a previous experiment showed that 0.5 c.c. of a 1/20 dilution of the serum in the presence of excess of complement completely haemolysed 0.5 c.c. of a 1/20 suspension of rabbits' red cells in 1 hour at 37°C. This serum will be referred to as serum 'β.'

Experiment.

A rabbit was selected and the fragility of its red cells determined. A red cell count was made and two blood films were prepared and stained with Leishman's stain. These last showed the scanty polychromatophilic cells normally present in the rabbit's blood. Another sample of blood was taken, allowed to stand for 30 minutes at 37°C., and the clot separated by centrifugalisation. It showed no trace of tingeing with haemoglobin. All these observations were repeated at intervals after the injection of 1 c.c. of Serum 'β' intravenously. After 46 hours, a second intravenous injection of 1 c.c. of this serum was given and the observations were continued. The results obtained were as follows :—

Fragility of Erythrocytes

Time of examination	Strength of saline solution causing first trace of haemolysis	Red cells per cmm.	Serum	Stained films
Before injection	0.56 %	5,340,000	Nil	Normal.
After injection				
1 hour	0.59	4,650,000	Nil	Normal.
4 hours	0.59	3,930,000	Nil	Normal.
24 "	0.63	—	Nil	Slight increase in polychromatophils and a few nucleated red cells.
46 "	0.58	4,360,000	Nil	Normal.
Further injection of 1 c.c.				
47 "	0.64	5,510,000	Nil	Normal.
52 "	0.63	—	Nil	Nucleated red cells more numerous.
55 "	0.68	4,600,000	Nil	Nucleated red cells numerous.
72 "	0.73	3,700,000	Very slight tingeing	Nucleated red cells very numerous.
100 "	0.72	3,680,000	Very slight tingeing	Nucleated red cells very numerous.
220 "	0.67	4,050,000	Nil	Nucleated red cells scanty.

The animal showed only slight signs of distress during the earlier part of the experiment and made a complete recovery. The results are shown graphically in Chart 6. The examination of the table, or better still the chart, shows several points of interest. It is clear that increased fragility has gone hand-in-hand with blood destruction; the lowest counts correspond with the highest saline values. There is also, however, a certain correspondence between the increase in fragility and the number of abnormal red cells present, so that we cannot, as a result of this experiment, exclude this factor. It became necessary, therefore, to perform further experiments in which the action of the serum should be less marked and slower and also in other cases more rapid and severe.

Experiment.

The rabbit was selected and the same preliminary examinations made as in the last experiment. 2 c.c. of Serum 'β' were injected intraperitoneally, and the resulting changes recorded at intervals.

Time of examination	Strength of saline solution causing first trace of haemolysis	Red cells per cmm.	Serum	Stained films
Before injection	0.56 %	4,560,000	Nil	Nil
After injection				
1 hour	0.59	—	Nil	Nil
4 hours	0.59	4,610,000	Nil	Nil
24 "	0.62	3,540,000	Nil	Nil
53 "	0.64	4,240,000	Nil	Nil
101 "	0.62	—	Nil	Nil

Here, too, the same general correspondence between blood destruction and increased fragility appears, while it is proved that an increase corresponding to 0.08% saline may occur without any abnormal red cells being present in the peripheral circulation.

In the following experiments a third haemolytic Serum ' γ ' was employed. It consisted in the mixed sera of two guinea-pigs, immunised as described above, but with somewhat larger doses of rabbits' red cells. It was heated before use, and was found to be slightly more powerful than Serum ' β ' as the result of titration.

Experiment.

Employing exactly the same procedure as in the two preceding experiments, a rabbit was inoculated intravenously with 5 c.c. of Serum ' γ .' The various examinations carried out gave the following results:—

Time of examination	Strength of saline solution causing first trace of haemolysis	Red cells per cmm.	Serum	Stained films
Before injection	0.57 %	6,050,000	Nil	Nil.
After injection				
5 mins.	0.63	5,000,000	Slight tinge	Nil.
20 ..	0.72	3,750,000	Deep tinge	Nil.
40 ..	0.72	3,500,000	Deep tinge	A few nucleated red cells.

Death occurred within the hour.

The occurrence of haemoglobinaemia of a marked degree caused the saline in every tube to be tinged with haemoglobin, so that it was necessary to work to a uniform pink-tinged fluid instead of to a completely colourless one. As it was possible, however, to arrange the series of solutions so that a long range beyond the saline corresponding to the maximum fragility was employed, a considerable number of tubes showing the exact tint due to the serum were obtained, and no difficulty was experienced in picking out the tubes in which the additional tinge due to hypotonic saline lysis was present. The results are shown graphically in Chart 7. This experiment entirely confirmed the results obtained in the two preceding ones, and points to the conclusion that the fragility increases directly with the blood destruction and is entirely unrelated to the appearance of abnormal red cells in the general circulation.

A second experiment was made, using this same serum, and inoculating intravenously, but with a smaller quantity, 2 c.c. The results are shown in Chart 8, and the full details need not be

EFFECT OF INTRAVENOUS INJECTION OF HAEMOLYTIC SERUM 'γ.'

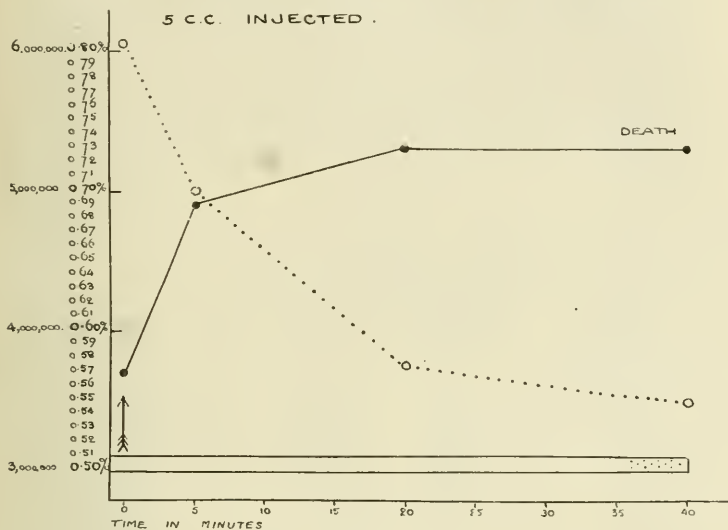


Chart 7.

EFFECT OF INTRAVENOUS INJECTION OF HAEMOLYTIC SERUM 'γ.'

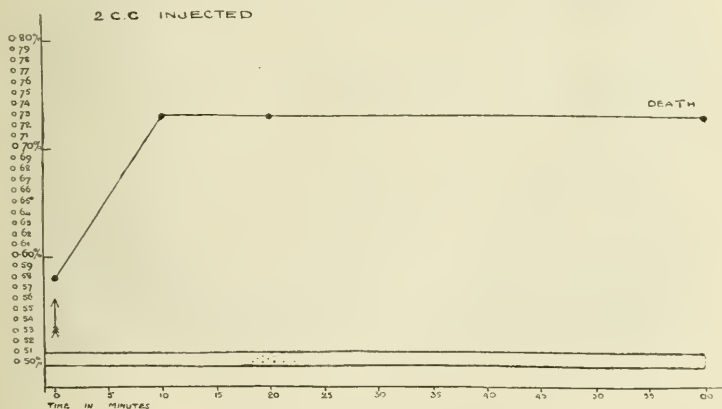


Chart 8.

tabulated here. The fragility rose rapidly, so that within 10 minutes its maximum value, which was originally equivalent to 0.58% saline became equivalent to 0.73% saline. Haemoglobinaemia was marked at this first examination and the animal died in little over one hour. At the 20 minutes' examination, stained films showed a slight increase in basophilia and the presence of a few nucleated red cells, but at each of the other examinations they showed nothing abnormal.

Finally, the same serum was employed using the intraperitoneal route.

Experiment.

The usual estimations were made and 2.5 c.c. of Serum 'γ' was then injected. The results were as follows:—

Time of examination	Strength of saline solution causing first trace of haemolysis	Red cells per cmm.	Serum	Stained films.
Before inoculation	0.56 %	5,000,000	Nil	Nil.
After inoculation				
½ hour	0.59	4,790,000	Nil	Nil.
¾ „	0.65	4,987,000	Nil	Nil.
1¾ hours	0.65	4,050,000	Nil	Nil.
2¼ „	0.62	3,900,000	Nil	Nil.
8 „	0.71	3,600,000	Nil	Nil.
22 „	0.58	—	Nil	Nil.
51 „	0.62	4,420,000	Nil	A few nucleated red cells.
95 „	0.56	4,987,000	Nil	A few nucleated red cells.

The animal never showed any marked sign of distress, and eventually made a complete recovery. The results are shown graphically in Chart 9. Here again there is the obvious correspondence between increased fragility and blood destruction, while abnormal cells only appeared on the scene when the fragility was returning to normal limits.

It would be an additional demonstration of the independence of the two phenomena, if we could produce marked abnormalities in the morphology of the peripheral blood without a corresponding alteration in fragility. This was attempted by making use of the well-known alteration which may be produced in the blood by repeated severe haemorrhages. The effect of a single severe, or repeated slight haemorrhages, had already, in a series of preliminary experiments, been found to be nil. There was a strong probability that a similar result would follow from repeated large abstractions of blood, since Theobald

Smith, first alone and later in conjunction with H. R. Brown, had already studied exhaustively the red cell fragility in a large series of horses, repeatedly bled for the purpose of obtaining anti-toxic sera. These papers have been referred to above and it will be remembered that these workers finally concluded that there was no evidence that any of the variations which occurred depended on the blood abstraction. The blood losses in this series of experiments, however, though severe, were not extremely frequent and there is no evidence as to their effect on the morphological characters of the peripheral blood, so that it was necessary to carry out a special series of observations bearing on this point.

EFFECT OF THE INTRAPERITONEAL INJECTION OF 2.5 C.C.
OF HAEMOLYTIC SERUM 'γ.'

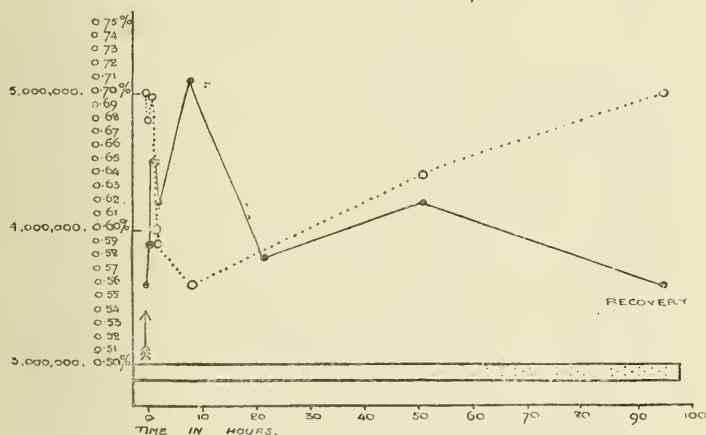


Chart 9.

Experiment.

A rabbit was selected and a preliminary determination made of its red cell fragility and of the morphology of its peripheral blood. It was then bled to large amounts, first on alternate, and then on successive days. Each day before the blood was abstracted the corpuscular fragility was determined, and two blood films were prepared. These

were afterwards stained by Leishman's method and any abnormalities carefully searched for. The results obtained were as follows:—

Day	Amount of blood extracted	Strength of saline solution causing first trace of haemolysis	Stained films
1st	10 c.c.	0·58 %	Nil.
3rd	20	0·55	Nil.
5th	55	0·56	Nil.
7th	40	0·55	Increased basophilia.
10th	40	0·58	„ „
11th	40	—	„ „
12th	10	0·56	A few nucleated red cells.
13th	5	—	„ „
14th	50	0·56	„ „
15th	—	0·55	„ „
16th	50	0·56	„ „
18th	50	0·55	Nucleated red cells numerous.
20th	—	0·56	Very few nucleated red cells.

The rabbit was somewhat distressed immediately after each of the larger bleedings, but the symptoms always passed off within a few minutes, and at the end of the experiment the animal appeared to be in perfect health. The results of this experiment are shown graphically in Chart 10. It is clear that the alterations produced are slight and of no permanent character, mostly falling within the limits of experimental error, and probably partly accounted for by the alteration in the tonicity of the serum which must result from the repeated and frequent abstraction of so large a proportion of the animal's total blood. Thus it is possible to cause the appearance in the peripheral circulation of the rabbit of a considerable number of morphologically abnormal red cells, without altering the maximum fragility to hypotonic saline, thus affording additional evidence of the complete independence of these two phenomena.

Having thus established the fact that a specific haemolytic serum is able to act on red blood corpuscles 'in vivo,' in such a manner as to increase their fragility towards hypotonic saline solutions, it becomes necessary to examine, as far as possible, the means by which this change is brought about.

A vast number of 'in vitro' experiments have yielded a considerable mass of precise information regarding the manner in which a specific haemolysin produces its effect, and a certain number of observations were undertaken in order to determine whether certain factors, which have been proved to be involved in the actual lysis of red cells, are concerned

EFFECT OF REPEATED BLEEDING ON THE FRAGILITY OF RABBITS' RED CELLS.

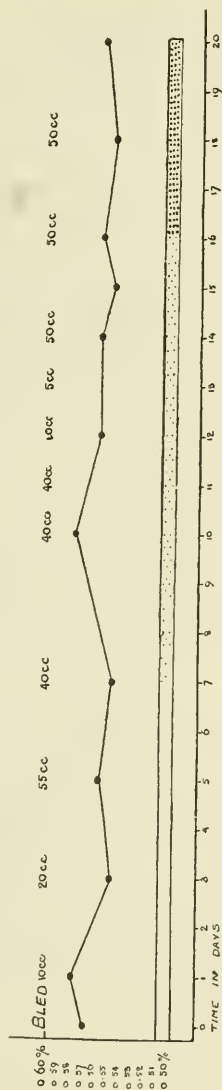


Chart 10.

also in producing alterations in their fragility. The first point investigated was as to whether the haemolytic amboceptor, apart from complement, had any effect on the corpuscular fragility. In the initial series of experiments human red cells were employed, together with the serum of a rabbit which had received a large number of intraperitoneal injections of washed human corpuscles. The human red cells were collected and treated in the routine manner, and the thick deposit from the last washing, during which centrifugalisation was carried out for a prolonged period and at high speed, was employed for the tests. The rabbit's serum was obtained in the usual manner and heated at 58°C. for 30 minutes to destroy complement. This serum, in addition to the specific haemolysin, contained an extremely powerful haemagglutinin, as is indeed usually the case in rabbit *v.* human haemolytic sera, and this fact rendered the preliminary treatment of red cells by the serum and their subsequent washing and testing impracticable, since the violence necessary to break up the firmly clumped red cells, in order to subject them first to washing and then to the action of the standard saline solutions, produced a certain degree of mechanical haemolysis which vitiated the result of the test.

The following method was therefore adopted. The requisite series of saline solutions were set up and then to each tube was added, first the usual amount of red cells, and then an equal quantity of the heated immune serum, or saline, or normal serum used as controls. The whole series were then placed in an incubator at 37°C. for 15 minutes and then centrifugalised as usual. The shorter time was adopted on account of the rapid settling of the cells in the tube containing the immune serum.

Experiment.

Contents of tubes	Strength of saline solution causing first trace of haemolysis
Standard Saline + Red Cells + Normal Saline	= 0.42 $\frac{0}{10}$
" " + " + Heated Immune Serum	= 0.40 $\frac{0}{10}$

Thus the red cells appeared to be less fragile in the presence of the heated immune serum containing the haemolytic amboceptor. This effect might have been due to the binding of the amboceptor to the red cells, but it was equally possible that it resulted from the mechanical action of the haemagglutinin in rapidly removing the red cells from the action of the saline, since clumps of massed corpuscles formed almost instantaneously in the tubes containing the immune serum. A third possibility was that the variation was caused by the difference between the tonicity of the serum and that of the normal saline.

Experiment.

The serum was obtained from a normal rabbit and heated as above to destroy complement. It was tested for the presence of a haemolytic amboceptor towards human red cells with a negative result. It was then tested for the presence of haemagglutinins by mixing one volume of the heated serum, one volume of washed human red cells and ten volumes of saline in a capillary pipette, and incubating at 37°C. for 15 minutes. Marked haemagglutination occurred. The following tests were put up with the results shown.

Contents of tubes	Strength of saline solution causing first trace of haemolysis
Standard Saline + Washed Red Cells + Normal Saline	= 0.41 %
" " + " " + Heated Immune Serum	= 0.40 %
" " + " " + Heated Normal Rabbit Serum	= 0.38 %

Thus, the heated serum of the normal rabbit which possessed no haemolytic amboceptor had a greater effect than the specific haemolytic serum.

To exclude the normal protective action of the serum the following series of tests were made.

Experiment.

The washed red cells taken from a normal human subject 'A' were used throughout. The sera employed were as follows:—

- (1) 'A's Heated Serum.
- (2) The Heated Serum of another normal human subject, 'B.'
- (3) The Heated Serum of a normal rabbit.
- (4) The Heated Immune rabbit Serum.

A preliminary test for the presence of haemagglutinins was made in the manner described above with the following results.

A's Serum and B's Serum produced no agglutination. The two rabbits' Sera as before agglutinated the red cells to a very marked degree. The following tests were then put up and the results recorded.

Contents of tubes	Strength of saline solution causing first trace of haemolysis
Standard Saline + A's Washed Red Cells + Normal Saline	= 0.40 %
" " + " " + A's Heated Serum	= 0.39 %
" " + " " + Heated Normal Serum	
	(Rabbit) = 0.37 %
" " + " " + Heated Immune Serum	
	(Rabbit) = 0.37 %

Another experiment employing a non-agglutinating human serum and an agglutinating rabbit's serum yielded corresponding results. It appears, from these experiments, that the amboceptor present in a rabbit *v.* human haemolytic serum has, by itself, no power of increasing the fragility of the red blood cells. The haemagglutinins present cause an apparent decrease in fragility, due probably to purely mechanical causes.

Nolf, however, in the communication referred to above, states that, whereas the normal erythrocytes of the fowl show no haemolysis in 0.45% saline, they may, when sensitized by a specific serum, show a faint trace of haemolysis in 0.55% saline or in even more concentrated solutions.

Turning now to the effect of a complete haemolytic system, amboceptor plus complement working 'in vitro,' the difficulties involved in the technique become very great. The most satisfactory procedure would obviously be to submit washed red cells to the action of both amboceptor and complement in amounts which fail to produce haemolysis, separate and wash the cells and then test them in the various saline solutions. The large number of washings and the considerable manipulation of the cells, involved in this process, have, however, a very appreciable effect. The amboceptor and complement, moreover, once bound to the cells, continue to act upon them and, thus, minute traces of these substances, which produced no trace of haemolysis during the usual incubation period, caused a slight degree of lysis during the somewhat lengthy operations which followed. Experiments in which the incubation was carried on for three hours, to allow the haemolysin to exert its full influence before the cells were washed, did not overcome this difficulty. The effect of carrying out all the operations, subsequent to incubation, as nearly as possible at 0° C. in order to prevent the further action of the haemolysin, yielded even less satisfactory results, since the effect of cold on the red cells was even more marked than it is on normal erythrocytes, which are bathed in hypotonic saline solution, and the fluid in all the tubes showed a strong tinge of haemolysis. For this reason, it is of little advantage to give full details of the experiments performed, but the general technique and results may be noted. Two combinations were studied (*a*) rabbits' red cells and a guinea-pig *v.* rabbit haemolytic system, (*b*) human red cells and a rabbit *v.* human serum. In all cases a $\frac{1}{10}$ dilution of normal guinea-pig serum was employed as complement and the haemolytic immune body was diluted to a point considerably above that which gave the last trace of haemolysis in

a preliminary experiment. The red cells were washed in the usual manner and a $\frac{1}{20}$ suspension prepared. Considerable quantities of such a suspension were subjected to the action of immune body+complement, immune body+saline, complement+saline, and a double volume of saline alone. Incubation was carried on for periods of one to three hours and the mixtures were then centrifugalised and the deposited red cells washed twice in normal saline. The deposit from the last washing was added to the test solutions in quantities of 0.05 c.c. to each tube. In almost all experiments there was, at any given dilution, most marked haemolysis in the tube containing red cells which had been subjected to the action of amboceptor+complement, the red cells which had been suspended in normal saline showed less destruction, while the tubes containing those cells which had been in contact with immune serum alone or complement alone, showed the least tingeing of the supernatant fluid. For this reason, a similar method to that employed when testing the action of amboceptor alone was resorted to.

Experiment.

Sheep's red cells were washed in the usual manner. Six saline solutions of different strengths were prepared, and with each of these a $\frac{1}{20}$ suspension of the red cells, a $\frac{1}{10}$ dilution of fresh guinea-pig serum and varying dilutions of a heated rabbit *v.* sheep haemolytic serum were made up. In every tube was then placed 0.5 c.c. red cell suspension, 0.5 c.c. complement and 0.5 c.c. diluted immune body. The whole series were then incubated for 30 minutes at 37°C. after which the tubes were centrifugalised and the degree of haemolysis noted. The results were as follows:—

Haemolysis produced by increasing dilutions of immune body.

Strength of saline solution	$\frac{1}{20}$	$\frac{1}{40}$	$\frac{1}{80}$	$\frac{1}{160}$	$\frac{1}{320}$	$\frac{1}{640}$	$\frac{1}{1280}$	$\frac{1}{2560}$	$\frac{1}{5120}$
0.85 %	Complete	Complete	Complete	Almost complete	Marked	Slight	Trace	—	—
0.83 %	Complete	Complete	Complete	Complete	Complete	Almost complete	Marked	Trace	—
0.81 %	Complete	Complete	Complete	Complete	Complete	Marked	Slight	Trace	—
0.79 %	Complete	Complete	Complete	Complete	Complete	Complete	Slight	Trace	Trace
0.77 %	Complete	Complete	Complete	Complete	Complete	Almost complete	Slight	Trace	Trace
0.75 %	Complete	Complete	Complete	Complete	Complete	Complete	Almost complete	Slight	Trace

This observation is, however, by no means a new one. As long ago as 1900, Nolf, in the article already referred to, showed that the haemolytic action of specific sera was retarded by the presence of certain metallic salts, and that this retardation increased with increasing concentration of the solution. In this connection he studied the action of the sera of the ox, dog, and rabbit on the corpuscles of the rabbit, horse, dog and fowl. As the result of a considerable number of experiments he found that the salts of the alkaline metals opposed haemolysis, in proportion to the concentration of the solution used, while the salts of the metals of the alkaline earth series inhibited the action of a haemolytic serum in all dilutions.

More recently, Sutherland and McCay have made similar observations, using anti-sera derived from rabbits, fowls and a goose, which had been immunised against sheep's red cells. They employed the chlorides of sodium and calcium, and found that the action of a haemolytic serum varied inversely with the concentration of the saline solution, while the presence of calcium salts had a most marked inhibitory action, thus confirming exactly the results obtained by Nolf. They could not obtain evidence that the haemolysin itself was affected, and assumed the difference observed to be due to variations in the resisting power of the erythrocytes. Nolf's hypothesis has been dealt with above.

Now it is obvious that experiments of the above type may be regarded as indicating either that the hypotonicity of the saline increases the fragility of the red cells towards the action of the serum, or that the action of the serum increased the liability of the corpuscles to hypotonic saline lysis. These considerations also involve a question as to the validity of the results obtained by injecting haemolytic sera into animals, since it might be argued that, in adding the blood of the animal as such to the various saline solutions, one in reality added both red cells and a minute quantity of the specific haemolysin, which might be considered to be present in the blood stream. Hence, the results might be obscured by a lytic action due to this trace of haemolysin in the hypotonic saline solutions. Various considerations, however, completely negate this idea. In the first place, the ultimate dilution of the haemolysin arrived at by injecting a small quantity, 1 c.c. for instance, of a haemolytic serum into the general circulation of an adult rabbit, and then withdrawing 0.05 c.c. of the animal's blood and adding it to 1 c.c. of saline, is a considerable one. The comparatively weak haemolytic sera employed would produce no lytic action in this dilution,

when acting for 30 minutes at room temperature. Again, if the action of this minute trace of haemolytic serum were a disturbing factor, it would obviously operate most strongly immediately after the injection, when it would be present in the highest concentration. As a fact, the effect with small or moderate doses rises to a maximum at some point between the second and fourth days. Finally, such an action would be a very gradual one, especially at room temperature; whereas, with alterations in fragility of the degree observed in the series of experiments in question, the change is obvious immediately one adds the blood to the saline solutions, by the partial laking of the corpuscles in tubes which before showed no such rapid lysis, although the particular tube which corresponds to a complete cessation of haemolysis can only be determined after centrifugalisation. It is thus clear, that the changes noted after the injection of haemolytic sera into rabbits correspond to an increased fragility towards hypotonic saline lysis, and this lends additional support to the assumption of Sutherland and McCay that it is the red cell itself and not the haemolytic system which is affected by the saline concentration in the 'in vitro' experiments.

CONCLUSIONS.

1. Of the various arsenical compounds studied (Arsenious Acid, Atoxyl and Salvarsan) none produced 'in vivo' any decrease in red cell fragility; while the two former in highly toxic doses produced a pronounced increase.
2. The injection into rabbits of foreign bile, of bile obtained from animals of the same species, of bile salts (Sodium Taurocholate) and of Cholesterin resulted in no change in fragility worthy of note.
3. Various pathogenic organisms known to be associated with haemolytic phenomena (*Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus* of Danysz and *Bacillus pyocyaneus*) produced in the majority of cases, when inoculated into rabbits, a rise in fragility of varying degree, most marked in the case of certain strains of *Streptococci*.
4. The injection into rabbits of specific haemolytic sera, heated or unheated, constantly produced an increase in fragility, in many cases of a most marked type.
5. This increased fragility was not due to the appearance in the peripheral blood-stream of cells of abnormal type, but resulted from the direct action of the haemolytic serum on the erythrocytes.

It was directly proportional to the degree of blood destruction which occurred.

6. The action of a haemolytic amboceptor alone did not, in the case of a rabbit *v.* human serum, produce any increased fragility. There was indeed an apparent decrease, but this was due to the mechanical action of the coexisting haemagglutinins.

7. 'In vitro' experiments on the effects of specific haemolysins on red cell fragility were unsatisfactory, on account of the extreme technical difficulties involved; but such evidence as could be obtained confirmed the 'in vivo' findings.

8. The relation between the action of the more specialised haemolysins and that of weak saline solutions, or distilled water, has never yet been definitely established; but the effect produced by the action of certain agents of one class results in an altered fragility to agents of the other.

It only remains for me to acknowledge my indebtedness to Dr L. S. Dudgeon for many kind and helpful suggestions.

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FIRST REPORT OF THE NORTH MANCHURIAN PLAGUE PREVENTION SERVICE.

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International Plague Conference, 1911.*

(With Plates VI-XVI, 1 Map, and 4 Plaus)

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I. INTRODUCTION.

IMMEDIATELY following on the International Plague Conference held in Mukden in April, 1911, the Chinese Government, anxious to carry out the recommendations of the Conference, instituted the North Manchurian Plague Prevention Service. The chief of these recommendations were briefly :

(a) Systematic investigations should be made as to whether epizootic plague occurs among Tarbagans and other rodents, and, if such exists, an accurate investigation should be made of the nature of the infection.

(b) A general improvement in the sanitary condition of cities and villages, especially with regard to overcrowding, is desirable.

(c) Education of the public by lectures and the issue of pamphlets and handbills, explaining preventive measures in simple language.

(d) The need for isolation of pneumonic-plague patients being urgent, permanent hospitals should be available.

(e) Hospital accommodation for suspected cases of plague should be provided.

(f) Contact quarantine stations should be constructed¹.

The primary object of the scheme was the formation for North Manchuria of a medical service equipped and ready to act and control any situation that might arise through an outbreak of plague in the district, and also to maintain hospitals at Harbin (the headquarters of the Service), Aigun (Taheiho), Sansing, Lahasusu, Manchouli, and any other town in the district where such an institution might be necessary. In addition medical service was to be given to the poor and destitute, and when the hospital accommodation was not required for plague cases, general medical, surgical and infectious diseases were to be treated in the wards. Further, medical officers in the Service were to instruct the people in general hygiene, etc.

It was hoped that the Service would develop into a Public Health Service for the whole of China. The funds for building the hospitals were to be derived from the current revenue of the three Manchurian Provinces, while the Service was to be maintained out of the Maritime Customs dues.

¹ Incidentally, it may be mentioned that the Chinese Government had already established isolation hospitals and segregation camps, each capable of holding from 400 to 2000 persons, at the important railway centres of Shanhaikuan, Mukden and Koupangtzu and at the sea-port of Yingkow (Newchwang). The director of the N. Manchurian Plague Prevention Service is consulting physician to these establishments.

The Revolution which broke out in October 1911, however, delayed matters; later on the Manchurian Customs, which hitherto had remained a separate entity controlled by the Chinese Government directly, were temporarily given over to swell the general Customs Revenue of China in order to pay the Loans and Boxer Indemnities.

It was not until September 1912 that arrangements were finally made to allot the sum of 60,000 taels (78,000 roubles) a year of the Chinese Customs Revenue of Harbin for the maintenance of the Service.

The Manchurian Plague Prevention Service was thus established on a firm basis on October 1st, 1912, although $1\frac{1}{2}$ years previous to that date, medical officers had been stationed at Manchouli, Harbin, Tsitsihar (capital of Heilungkiang Province), and Lahasusu to carry out the objects for which the Service was founded.

In the preparation of this Report, I have received considerable assistance from my staff, particularly Dr S. P. Ch'en, M.B., B.C. (Cantab.), Senior Medical Officer at Harbin, and Dr F. E. Reynolds, M.B., Ch.B. (Edin.), Bacteriologist of the Service.

Among the delegates of the International Plague Conference, Mukden, April 1911, it was generally believed that there was a close connection between Plague—or at least the recent epidemic of Pneumonic Plague of Manchuria—and the Tarbagan or Marmot (*Arctomys bobac*, Schreb.).

So little is known about this animal and the grounds for associating it with Plague were based on such slender scientific evidence—as will appear later in this Report—that it seemed to me the first step in the investigation of this question was to organise an expedition into the country where the Tarbagan abounds, and there to study its habits and the conditions under which it lives and to carry out scientific investigations as to the presence of Plague among the animals.

For the sake of clearness, it is well to state here that the Tarbagan hunting is divided into two seasons:

I. Spring season, lasting from the end of April to the beginning of June.

II. Autumn season, lasting from the middle of August to the end of September.

The subject, however, will be dealt with in greater detail later in this Report.

On the morning of May 26th, 1911, I arrived at Manchouli to make enquiries as to the conditions under which hunting took place, and to gather information which would prove useful in making arrangements

for an expedition into the Tarbagan country later in the year. My observations made at that time are embodied in this Report. I left Manchouli on the evening of May 29th and returned to Harbin. About this time of the year large numbers of coolies arrive in Harbin principally from the province of Shantung to find work in the north. Most of them obtain employment along the Sungari and Amur rivers. On May 30th I saw a train reach Harbin station with over 1000 coolies; like others from Shantung, they possessed a fine physique and were in the best of health. Soon afterwards official business took me to Peking.

In the beginning of July rumours were afloat that a large number of Tarbagans were dying in the neighbourhood of Scharasone (Transbaikalia, Siberia). On July 11th I left Tientsin with orders to organise an expedition to investigate the matter, as the Chinese Government were most anxious that all measures should be taken to forestall a possible spread of Plague to man. The Manchurian authorities agreed to build hospitals for Plague at all the important centres and instructed me to staff them with properly qualified medical officers; and from the Viceroy at Mukden downwards, all the officials took great interest in the proposed expedition. I quote the following from the *Peking Daily News*:

Tarbagan Epidemic in Siberia spreading southwards.
(Special Dispatch to the *P.D.N.*)

HARBIN, July 13th.

"My correspondent at Station Manchouli wires that the Tarbagan epidemic of Siberia is spreading southwards towards the Chinese frontier. Dead marmots are now found as far south as Scharasone which is only thirty-five miles north of the Russian-Chinese frontier. Local measures are sufficiently strict to prevent the disease from entering Manchuria. Some members of the Chinese Scientific Expedition for the study of Tarbagan diseases have already arrived here."

Arriving at Harbin on July 15th, I called on Professor Zabolotny, who was at that time working at certain plague problems in the Russian Laboratory of that town. After showing me his specimens and telling me the results of his investigations—a note of which appears in this Report—he invited me and my party to proceed with him to Manchouli in a special car provided by the Chinese Eastern (Russian) Railway. I gladly availed myself of the invitation. It is interesting to note that this was the first scientific expedition sent out by the Chinese Government.

The Russian party consisted of Professor Zabolotny, Drs Tschourilina (a lady) and Issaief (see Pl. VII, fig. 3). Our own party consisted of Dr Ch'en Sze-pang, M.B. (Cantab.), Dr Tsang Pu and myself. Our investigations form the basis of this Report.

II. ITINERARY.

Leaving Harbin we arrived at Manchouli Station on July 21st and arranged that this should be the base of the Chinese Expedition. We gave instructions that huts should be built for us, for the laboratory, and for the animals. The next day Dr Ch'en and myself left for Borsja¹ (Transbaikal District) to join the Russian party who had their base there. We remained with them for a week, visiting Tschintansk, Arabulak and the neighbouring villages. On July 29th we returned to Manchouli to rejoin our party. A few days afterwards the Russian staff broke up, Prof. Zabolotny and Dr Tschourilina returning to St Petersburg and Dr Issaief to his station on the Russo-Chinese frontier. Our start for Mongolia to make further investigations was delayed by the rainy weather until August 6th. Proceeding westward we reached Charbada on the 7th, where we encamped for two days. On August 9th we left for Kerloni. As there was no direct way there we had to return to Manchouli and from thence proceeded in a south-westerly direction parallel to the large but shallow river called Kerulen, which after it has passed through Kulun Nor (Kulun See) is called River Argun, and later on becomes the Amur. We travelled southwards, passed Zagan on the east, and after two days reached the large lake, Kulun See. Finally we encamped at Kerloni—a Mongol colony situated on the river—on August the 11th. We then proposed to visit Abagaitui in Siberia by way of Dalai Nor, but our guide informed us that this was impossible, as it would mean crossing a mountainous district where there was no water. On the following day, therefore, we decided to return to Manchouli, which we reached on August 14th. From this date until August 25th we lived at Manchouli, making excursions into the country around and doing experimental work with Tarbagans.

On August 25th I departed from Manchouli in order to enquire into an outbreak of Plague which was reported from Puk'uei (Tsitsihar, capital of Heilungkiang Province) and from thence went back to Harbin. I left Dr Ch'en at Manchouli to finish our work there and

¹ Borsja is 121 versts west of and three hours by express train from Manchouli.

to make any other investigations that might be required. Dr Ch'en's work at this time is incorporated in this Report. He left Manchouli for Harbin on September 30th, 1911, as the evenings were getting too cold for further stay in camp.

III. MANCHOULI¹.

The principal marts in the Manchurian Provinces for Tarbagan skins are Manchouli and Hailar. Hailar is 116 miles (174 versts) by rail east of Manchouli station, but as the latter is the only Customs station for the district, skins sold in Hailar have to be sent there for exportation. The chief Russian mart is Borsja (Transbaikalia), some 80 miles (121 versts) west.

Manchouli is situated in Chinese territory at the junction of the Chinese Eastern and the Trans-Siberian Railways, and is about ten miles east of the frontier between the Province of Heilungkiang and Siberia. Here are established the Chinese and Russian Customs for the examination of goods and baggage passing along the railway from one country to the other—Manchouli having been opened as a Customs station in February 1907. Most of the information which follows about the place was obtained when I visited the town at the end of May and again in August.

Manchouli (meaning the hamlet of the Manchus) lies 2600 feet above sea level and is surrounded on all sides by low barren mountains. The water is saltish, hard and scarce. The railway zone covers a large area and consists of four sections:

(1) The Private Settlement containing 5000 Russian and 1500 Chinese inhabitants.

(2) The Chinese Eastern Railway Settlement containing about 700 inhabitants, mostly Russians.

(3) The Transbaikal Settlement containing 800 inhabitants.

(4) The River Settlement on the further side of the "river"—a shallow ditch some five feet wide. This Settlement used to be occupied by desperadoes and murderers, mostly Caucasians, who carried on a trade in smuggling. It is now in ruins, having been destroyed five years ago by the Russian police who, after a three days' fight, caught and exiled the survivors to Siberia.

Altogether the population of Manchouli may be estimated as consisting of 8000 Russian civilians, Customs officials, etc., 3000 Russian

¹ Manchouli is 875 versts or 583 miles by rail west of Harbin.

troops, and 2000 Chinese, most of whom are of the coolie class. During the Russo-Japanese War there used to be as many as 40,000 to 50,000 people resident at Manchouli.

Occurrence of Plague at Manchouli and surrounding districts.

In 1898 Prof. Zabolotny was in Mongolia and found Plague in the region he visited. Between this date and 1905 I could gather no reliable information as to the occurrence of the disease in or around Manchouli. Dr Bissemsky, the Russian physician in charge of the railway at Manchouli, supplied me with the following interesting information regarding outbreaks of Plague which had occurred in his district since he went there in 1905:

- 1905. Plague occurred in August at Dalai Nor¹ (14 cases) and in Manchouli (4 cases). Total 18 cases. Bubonic type.
- 1906. Plague occurred in Abagaitui² (Russian territory) (15 cases) and in Manchouli (2 cases). Total 17 cases. Pneumonic type.
- 1907. One case was imported from Transbaikalia territory into Manchouli. Bubonic type.
- 1908. There were no cases of Plague in Manchouli, but there was reason to believe that the disease was present among the Mongols along the Argun River. There is some doubt as to the type of the disease, but it seems probable that it was pneumonic.
- 1909. No cases reported.
- 1910. The last epidemic, with 400 cases at Manchouli. Pneumonic type.

To bring it up to date, the above information may be supplemented as follows:

- 1911. End of August, 5 cases at Scharasone, 4 deaths. Bubonic type.
- 1912. Beginning of September, near Chita³ (capital of Transbaikalia), 3 cases, all fatal. Evidently pneumonic type (confirmed by P. Haffkine).

In regard to the above outbreaks, it is interesting to note that those of 1905, 1906, 1907, 1911 and 1912 were confined to Russians and Cossacks, no Chinese having died: whilst in 1908 only Mongols died.

¹ Dalai Nor is the first railway station, 28 versts east of Manchouli.

² Abagaitui is 20 miles north of Manchouli.

³ Chita is 447 versts N.W. of Manchouli.

In the epidemic of 1910-11 both Russians and Chinese were attacked, but mainly the latter; of 400 fatal cases at Manchouli only about 15 were Russians, and these were mostly hospital attendants. For some reason which we cannot explain, during the epidemic of 1910-11, the Russians escaped even when some of them lived with Chinese coolies who took the Plague. In considering the occurrence of Plague at and around Manchouli, it must be borne in mind that the disease is endemic in the Kirghiz Steppes¹, and from time to time this has travelled eastwards giving rise to sporadic outbreaks in Eastern Siberia.

This subject has been treated carefully in a series of articles written by the British Delegate to the Constantinople Board of Health² and by the Russian, Dr Koltshof³, quoted by the British Delegate. So important is the subject in reviewing the occurrence of Plague in Manchuria that I quote these articles at some length. For convenience the subject has been considered more or less in chronological order.

Koltshof gives the following summary of the epidemics in the Bukeef Horde of the Kirghiz Steppes of the Astrakhan government during the twelve years preceding 1911. The disease generally gives rise to two annual epidemics—one in summer, the other in winter.

- 1898. Up to this year there are said to be no records of Plague in the Bukeef Horde.
- 1899. Summer, no outbreak. Winter, 1899-1900, epidemic began on November 15th.
- 1900. Summer, no outbreak. Winter, 1900-1901, epidemic began on December 23rd.
- 1901. Summer, no outbreak.
- 1902. Summer, epidemic began on June 3rd.
- 1903. No Plague in the Horde, though epidemic elsewhere in the Astrakhan government.
- 1904. In December, imported from Kirghiz and Cossacks in the Uralsk Province, where 415 persons had died.
- 1905. Summer, some minor outbreaks. Winter, an exceptionally severe epidemic. From October 13th to February, 1906, some thousand persons lost their lives.

¹ The Kirghiz Settlements are situated in the governments of Astrakhan (Europe), Uralsk and Semiretchinsk (Asia). The area covered by these three governments is a very large one.

² *Lancet*, April 24th, 1909, Jan. 7th, 1911, June 3rd, 1911, March 9th, 1912.

³ *Pratch*, No. 35, August 27th, 1911, quoted in *Lancet*, March 9th, 1912.

1906. Summer, epidemic began on April 15th. Winter, epidemic 1906-7 ended on February 28th.
1907. Summer, epidemic began on July 26th. Winter, epidemic began on October 11th.
1908. Summer, epidemic began on July 7th and ended August 3rd. Winter, no outbreak.
1909. Summer, no outbreak. Winter, 1909-10, epidemic began end of October and subsided in February.
1910. Summer, epidemic began on June 15th.

In Odessa, from May 22nd to August 28th, 1910, there were 97 cases of Plague of which 24 were fatal; and at the beginning of October, 1910, there were four cases.

In July, 1910, Plague appeared in the Kirghiz Steppes, namely in the Asiatic province of Semiretchinsk and in two villages of the Abbastin quarter in the Prjevalsk district of that province, there were 17 cases of pneumonic plague, all fatal, between July and August 11th. No further information regarding this outbreak has been published.

In the Uralsk government, in the five villages of the Jambaitinsk district, between August 10th and 14th, there were seven cases of plague, with three deaths. It should be noted that the dates given above are according to the Old Style calendar, which is 13 days later than the Gregorian.

Concerning the outbreak of pneumonic plague at Manchouli in the autumn of 1910, the following occurs in Dr Ch'uan Shao-ching's paper read at the International Plague Conference¹:

"Observations reported to me by Chinese residents (at Manchouli) show that two carpenters who lived in the house adjoining Wu Kuei-ling's inn died with spitting of blood on the 10th day of the 9th Moon (October 23rd). These two carpenters had been in the service of a foreman named Chang Wan-shun at Dawoolya (Daurija), a railway station in Siberia situated some six miles west of the boundary line. Chang Wan-shun told me that six or seven of his carpenters had died with blood spitting in Dawoolya on the 13th day of the 8th Moon (September 26th) and said he believed that plague had appeared in Dawoolya before it was known in Manchouli.

"Later on it was found that nine out of twenty coolies, who lived in a small room in Wu Kuei-ling's inn, were suddenly taken ill with blood

¹ *Report of the International Plague Conference*, p. 28.

spitting. One of them was sent to the Russian Plague Hospital, and it was discovered that he suffered from pneumonic plague. Two died in the house that same night, but the rest ran away to different places in the town, and thus disseminated the disease."

Returning to the articles already referred to in the *Lancet*:

"On October 14th, 1910, 17 deaths from pneumonic plague occurred in the village of Akurai in the district of Chita. During the week ending October 28th, 1910, there were four deaths at Daurija station, two cases at Tarbagatui mines near Petrovsk, and 34 cases at Manchouli.

"In the early part of 1911, Plague was still prevalent at the Kirghiz Steppes. In and near Sartube (in the first Maritime District), there were 31 cases with 29 deaths between January 4th and 20th. At Djaltir, also in Astrakhan government, seven persons in one hut fell ill with plague (February 3rd to 7th) and three died.

"During the latter part of 1911, outbreaks of plague of greater or lesser extent occurred in various Kirghiz settlements, the disease being particularly active in the Astrakhan government. In the Semiretchinsk government, which is situated close to the Mongolian frontier and to the north of Kashgar¹, plague appeared on August 31st (Old Style) in the Kirghiz settlement Akskoe in the *volost* of Teherikof. By September 14th there had been four cases, all fatal, of pneumonic plague. From Prjevalsk, on September 14th, were reported eight fatal cases of the same disease at a spot near Maryn in the same *volost*, and two more deaths occurred there on September 16th. By September 20th the epidemic was declared to be at an end.

"In the Uralsk government, situated much more to the west and adjoining the Astrakhan government, five deaths from pneumonic plague occurred in the Uletinsk *volost* before August 10th. In the same district, in the Turkoman settlement, near Djambent, eight fatal cases of pneumonic plague were recorded between June 29th and August 8th. In the Tchadyrtinsk *volost* of the same government, eight cases and three deaths from plague of the 'pneumonic and abdominal forms' occurred before September 10th, followed by one death on the 12th, and one fatal case on the 13th. Another case occurred in another settlement five *vershs* away, and the totals by September 16th had reached ten cases and six deaths.

"In the Astrakhan government, plague was epidemic in the Urotchishehe of Saganai from the end of August to middle of September; a score or more cases occurred here. On September 21st the disease

¹ Capital of Chinese Turkestan.

became epidemic in several other centres. The following table is a summary of the returns:

Period	No. of infected centres	Cases	Deaths
September 21st—Nov. 7th, 1911	16	73	63
“ “ — “ 21st, “	28	120	102
“ “ — “ 28th, “	33	139	119
“ “ — Dec. 19th, “	38	187	166
“ “ — “ 26th, “	43	201	180

“The infected centres were situated principally, if not entirely, in the Marynsk, the Kamysk-Samara, and the Primorskaia (or Maritime) divisions of the Astrakhan Steppes. The disease was most active in the Ak-tchagyl settlement (15 versts from the above named Saganai), and in those of Sarhube, Kudaunsk, Djapalatka, and Autantchagyl. Elsewhere but few cases or deaths occurred in any single centre. While many of the outbreaks were pneumonic in character, this was not invariably the case¹.”

“From Oct. 1911 to Feb. 1912, over 200 cases occurred in the Kirghiz Steppes; among the deaths from plague in the Astrakhan government were those of Dr I. Deminsky, a bacteriologist, and his student assistant. In the port of Kherson on the Black Sea a plague-stricken rat was found in September on a ship which had arrived from the port of Odessa, and this gave rise to a suspicion that the latter port had not yet got rid of the epizootic which was formerly reported among its dock rats.” *Lancet*, Dec. 28th, 1912, p. 1811.

PROHIBITION OF TARBAGAN HUNTING.

During February 1911, the (Chinese) Prefect of Manchouli prohibited the hunting of the Tarbagan, the penalty for disobeying this order being two months' imprisonment. In the following April a further order increased the punishment to six months' imprisonment (Appendix III). Up to June 1911 several Russian and some twenty Chinese hunters had been arrested and since then no one had been found hunting. The Chinese suffered imprisonment, and the Russian hunters were handed over to the Russian authorities. The punishment meted out to these Russian hunters is believed to have been confiscation of traps and of half of the skins found, the other half being returned after disinfection to the offenders.

On August 11th, 1911, a General Order, No. 26, was issued by the Russian authorities forbidding trade in Marmots, including hunting of

¹ British Delegate to Constantinople Board of Health, *Lancet*, March 9th, 1912.

animals, preparation of skins, salting of the flesh and fat under penalty of a fine of 500 roubles or three months' imprisonment. From enquiries made from the Russian police at Manchouli on August 21st it appeared that they had read of the Order but up to the time had not received official notice of it from the Head Office in Harbin. Consequently they were not empowered to put any hindrance on the trade in Marmot skins, although since the beginning of the year they had required, before granting permission to export, a formal guarantee that the raw skins had been sent to the local railway doctor for disinfection. How this disinfection had been carried out, or if it could be carried out at all, without spoiling the skins, was not known.

Up to 1908, comparatively few Chinese hunted the Tarbagan. In the autumn of that year and in the autumn of 1909 several thousand Chinese, attracted by the high price paid for the fur, were in the neighbourhood of Manchouli, and in 1910 the number reached 10,000 hunters.

Owing to the Chinese Order prohibiting the hunting of Tarbagans, there were fewer Chinese in Manchouli during 1911 than formerly, and in consequence the low class so-called lodging-houses, which used to be occupied by the hunters, had been left almost empty. The camping grounds just outside the town where the Mongol hunters used to erect their tents during the hunting season were also deserted.

THE INNS.

The inns are of two kinds—those entirely underground and those partly overground and partly underground. The overground dwellings are brighter since they are more open, by means of windows made either of glass or paper, to the sunlight, but those partially underground are more popular in cold weather because of their greater warmth¹. The windows of the latter appear just above the ground and their roofs are made of mud. The accommodation in both types of inns is based upon much the same plan. Two, and sometimes three, tiers of berths for the lodgers are present, there being just room enough between the tiers for a man to sit up. In one house I visited there were 40 berths—20 above and 20 below—the measurements of the room being only 2.5 ft. square and 14 ft. high. Even in May the rooms were ill-ventilated and stuffy. In winter when all the windows are closed and the fires are lit

¹ In winter the outside temperature as a rule reaches -30 to -40° C., and the ground is frozen to a depth of over 6 to 8 feet.

the stuffiness must be great. Dr Ch'uan visited some of these inns in the winter. His report showed the conditions to be bad in the extreme: in one hut, 15 ft. square and 12 ft. high, there were packed in three tiers of berths, one above the other, more than 40 people. The windows were closed and there was a heating stove in the middle of the room. The odour was indescribable, being made up of a mixture of foul breath, the vapours of old dirty fur garments and decomposing pelts which were lying alongside the men (Pl. VI, figs. 1, 2). The usual way of heating these inns is by means of a *k'ang* on either side of the central passage leading from the door. A *k'ang* is a horizontal brick flue about 5 ft. wide and 2 ft. high, at one end of which is an opening where is lit a fire of wood or *Kaoliang* (millet) stalk. At the other end of the *k'ang* an opening is made through the outside wall of the house, and leads to a chimney for the escape of the smoke. *Kaoliang* stalk is cheaper than wood and hence is usually used in poor houses; the heat produced is not very great but is sufficient to keep the room at 2 to 5 C. even when the thermometer outside registers -20°C . The windows are few in number, and usually made of a wooden framework pasted with white paper, thus keeping in the heat but allowing little sunlight to penetrate. The men eat, sleep, and very often cook in the same room. They sleep in rows with the head against the wall and the feet towards the central passage. There is no partition between adjoining berths so that they can easily breathe and cough into each other's faces. Their food consists mainly of millet bread, boiled cabbage, and boiled turnips; they have meat perhaps once a month. For board and lodging the hunter pays at least fifteen roubles a month; in busy times, more. The winter clothes usually consist of a pair of padded cotton trousers, padded socks and thick-soled Chinese shoes, two or three layers of body clothing and a long cotton-padded gown reaching to the ankle. In very severe weather they wear in addition long sheepskin garments and fur caps. During winter the men seldom change their clothes which abound with insects, *e.g.* lice, fleas, but in summer they lay aside their thick outer garments and wash the inner ones, using them alternately. Unless very poor, the winter trousers are then replaced by thinner ones which cost about a rouble (2s. 1d.) each.

In these inns I have found coolies from Shantung and Chihli, Manchus and Mongols; in addition, the hunters consist of Buriats and Cossacks. These unhealthy huts are owned by Russians and were built originally for their poor class nationals, but perhaps they were never so overcrowded as when Chinese occupied them.

From 1908 to 1910, so many people came to the districts to hunt the Tarbagans that the owners of the inns did a roaring business and packed the men in every available space, extra tiers being added whenever possible above those already existing.

It should be remarked that the number of Mongols regularly resident at Manchouli seldom reached a dozen; but at certain times of the year, especially in summer, large caravans consisting of whole Mongol families pitched their tents on the vacant space in front of the *Yâmen*¹. At the time of my visit in July 1911, there were nearly fifty families (some 150 people in all) thus encamped. Some lived in tents, others inside their rickety carts. These vehicles have no springs and no metal rims to the wheels. On such occasions the Mongols brought with them for sale horses, cattle and sheep as well as skins. They were very orderly and willingly abided by the rules laid down by the Prefect.

At Manchouli there is a General Hospital under the charge of the Russian doctor Bissemisky. This hospital has accommodation for 40 in-patients as well as separate blocks for infectious diseases and plague. There are besides a post-mortem room and quarters for the attendants.

Proposed Chinese Hospital at Manchouli.

Soon after the International Plague Conference it was decided to build a plague and a quarantine hospital at Manchouli, and the necessary funds amounting to Taels 40,000 (about £5600) were sanctioned by the Central and Manchurian Governments in May 1911—these funds coming from the surplus of the Plague Prevention Funds which had been voted during the epidemic of 1910–11. The hospital was to have accommodation for 20 plague cases, 20 suspect cases and 150 contact cases, and was to be in charge of a Senior Medical Officer with European degrees resident permanently with a trained staff. Plans had been submitted and selected and the foundations had been dug out in a vacant piece of ground near to the *Yâmen* of the Prefect. As Manchouli was so isolated, it was proposed that the bricks should be made locally. By October, 1911, the stones and timber had been carted to the site and a well dug. Winter setting in necessitated the work being stopped temporarily. Soon after came the Revolution in China, followed in January 1912 by the troubles in Mongolia. Among the things destroyed at Manchouli by the Mongols were the schools, the *Yâmen*, and even the foundations and materials intended for the new hospital.

¹ A *Yâmen* is a Chinese official's residence or place for transacting official business.

Note on the Fur Trade at Manchouli.

The Tarbagan hunting is divided into two seasons:

(1) The Spring season lasting from the end of April to the beginning of June.

(2) The Autumn season lasting from the middle of August to the end of September.

The early Spring fur, owing to its being lighter in weight, is more valuable than the heavier fur obtained in the Autumn season. The fur of the Tarbagan is thick, soft and very serviceable in winter; but up to five years ago the export was not great. Then came a big demand from Europe and America, for the fur dealers in London and Leipzig found that if properly cured and dyed, these furs could be turned into imitation sable and seal skins. The following are the Chinese Customs Returns showing the number of skins exported from Manchouli on which duty was charged during the years 1908-1912:

Pieces of	1908	1909	1910	1911	1912	Value in Customs Taels for 1912 (1 Customs Tael = Rouble 1.60)
Fox skins	407	3,502	4,378	638	1,274	Tls. 5,096
Goat skins	3,770	2,454	4,829	1,929	1,445	—
Sable skins	920	9,039	4,729	66	62	„ 1,530
Sheep skins	238,479	214,104	193,147	49,689	135,278	—
Squirrel skins	26,234	12,983	29,043	3,080	102,312	—
Tarbagan skins	210,224	19,181	242,458	10,673	55,196	„ 15,769
Total no. of furs	480,034	261,263	478,584	66,075	295,567	
Percentage of Tarbagan skins to total no. of skins	44 %	7.3 %	50.6 %	16 %	18.7 %	

The above figures, however, do not give an adequate idea as to the actual total export of furs from Manchouli, since many skins, as well as hides and bristles, were consigned to Mazijewsskaia, the first station on the railway west of Manchouli and within the 50 verst¹ duty free zone². Duty was not collected on these furs, the excuse being made that they were for use within the 50 verst free zone. It is estimated that in 1910, two million Tarbagan skins in all were exported from Manchouli. In June 1913 over four million Tarbagan skins were lying at Manchouli ready to be carted by road to Mazijewsskaia for export to Europe. In

¹ 3 versts = 2 miles.

² By the Treaty of 1881 made between China and Russia, it was agreed that within a zone of 50 versts on either side of the frontier between the two countries, goods shall be admitted free of charge.

weight it is estimated that 1000 Spring Tarbagan skins weigh 8 Russian *poods* (288 English pounds); whilst 1000 Autumn skins weigh 14 *poods* (504 English pounds), counting a *pood* as 36 pounds. The price rose rapidly from 15 kopecks to 50 kopecks per skin until in 1910, 80 kopecks per skin were realised.

Owing to the increased demand for skins and the consequent rise in price large numbers of coolies—and especially those from Shantung—were attracted to the spot, and the agricultural peasants left their fields to become Tarbagan hunters. By three months' hunting of the Tarbagans they were able to make as much money as they could have done in the course of a whole year from other occupations.

The Tarbagan skins are mostly sent to Leipzig and London to be cured. At the former town there is a large well-equipped factory for converting the skins into imitation-sable. Of late years, for greater safety, valuable furs have been sent to Europe by parcel post.

IV. PROFESSOR ZABOLOTNY'S WORK AT HARBIN.

When I visited Harbin in May and again in July, I called on Professor Zabolotny who was working there at certain problems in connection with plague. I believe that a full account of these has been published already, but it is not available to me. As some of his results and the information he had received influenced to a certain extent our future plans, it seems wise at this point to refer briefly to his results as given to me at that time:

I. He confirmed the fact that the cultures obtained from the donkey at Mukden were *Bacillus pestis* (see *Report of the International Plague Conference*, pp. 143-4).

II. His experiments on the infectivity of animals had given interesting results, viz.:

(a) Two donkeys, inoculated subcutaneously with cultures of *Bacillus pestis*, took plague badly but did not die¹. These were later on allowed to inhale large numbers of the bacilli through tracheotomy wounds, but both animals resisted the disease. When I saw these animals they were apparently quite healthy and playing about in the field.

(b) Pigs were successfully infected and died.

¹ Cf. Strong and Teague. From experiments performed by them, they consider that donkeys are *not* susceptible to pneumonic plague infection (vide *Philippine Journal of Science*, Sec. B, Vol. VII. pp. 225-7).

(c) Dogs took the disease but did not die.

(d) Other animals took plague easily.

(e) Birds were immune.

III. Zabolotny had obtained most interesting results from experiments conducted on 18 monkeys. He tried to protect them with vaccines and serum in large doses, but only two were saved, and these had received extraordinarily large doses. For instance he carried out the following series of experiments:

	Monkey <i>a</i>	Monkey <i>b</i>	Monkey <i>c</i>	Monkey <i>d</i>
1st injection (emulsion of dead agar culture)	1 c.c.	4 c.c.	5 c.c.	25 c.c.
2nd " " " "	2	5	10	30
3rd " " " "	3	5	10	—
Result after inhaling <i>B. pestis</i> through tracheotomy hole	Died	Died	Died	Ill for 1 week, then recovered

IV. If monkey *d*'s example is one to be followed for man, it would mean that at least two doses of 250 c.c. and 300 c.c. respectively of the emulsion would be required to produce immunity (taking a monkey's weight to be 6 kilos and the weight of a man 60 kilos). Three monkeys were also treated respectively with 100 c.c. of serum prepared in (a) Kolle's Laboratory, (b) the St Petersburg Laboratory and (c) L'Institut Pasteur in Paris. Only one survived the inhalation experiment, namely, the one treated with the Paris serum.

In a series of experiments, strains of *Bacillus pestis* from Mongolia (1898), Odessa and Bombay—all bubonic cases—were used for inhalation experiments. Death from pneumonic plague resulted, thus proving the similarity of the micro-organism in both types of the disease.

In another series, the experiments were reversed. Strains from Harbin and Mukden (pneumonic cases) produced the bubonic type of plague when inoculated subcutaneously.

V. Some weeks previous to my visit in July, Zabolotny had persuaded the authorities to allow cremation of all bodies dead from Plague which had been buried at Harbin during the previous winter, including those of the Doctors and Sanitary Staff. From 15 of these he removed the heart's blood for cultures and examination purposes. Results positive for *Bacillus pestis* were obtained in 10 out of 15 cases, including those of three doctors and two students. The soil surrounding the coffins, 2½ metres deep, was still frozen. These bodies were afterwards cremated and the ashes laid back again.

VI. A Tarbagan caught on June 24th by Dr Issaief near Scharasone on the Siberian side of the border was suffering from

Plague, and died soon afterwards. Zabolotny showed me the organs of this animal. There were marked signs suggesting Plague in the spleen, lungs (hæmorrhages) and there were cervical buboes. Growths obtained from the organs gave pure cultures. From one of the cultures thus obtained, Zabolotny inoculated in Harbin a healthy Tarbagan on the right leg and produced two large buboes in the right groin only. This animal died of septicæmia.

VII. Four other Tarbagans were suspected to have died of Plague. These had been found:

- (a) near Scharasone also by Issaief and Zabolotny,
- (b) at Arabulak south of Charbada (Mongolia) by Bissemsky,
- (c) at Kulussutai in Transbaikalia by Krestovsky,
- (d) at Borsja (121 versts west of Manchouli) by Bissemsky.

At the time when I met Zabolotny the diagnosis of these four cases was not complete. Later, however, it was found that none of the cases were Plague.

VIII. During July, Zabolotny had received reports of large numbers of Tarbagans dying north of the River Borsja in the neighbourhood of Kerulen. We decided to equip a joint scientific expedition and visit the spot without delay.

V. WORK AT BORSJA. July 22nd-29th, 1911.

(Plate VII.)

Borsja (Siberia) is 121 versts west of Manchouli on the Railway and is the centre of Tarbagan hunting in the Transbaikal district. During our stay there, no hunting was being carried on as this had been forbidden. There were some 200 to 300 Chinese engaged in small business; usually these depended on the Cossack hunters.

On the morning of July 22nd we arrived at Borsja, and joined Professor Zabolotny and his staff. The accommodation consisted of three railway cars:

- (a) a car fitted up for our living quarters;
- (b) a laboratory car fitted up with incubators, sterilisers, etc., and having a compartment in which post-mortems on animals were to be performed;
- (c) an animal car containing about 40 cages in which Tarbagans could be kept for observation. These cages were mostly oblong and were made of thin iron sheets and ventilated by means of round apertures punched in their sides.

Special permits to hunt were given to skilled Russian hunters who were specially instructed to bring in any sick Tarbagans. For healthy animals we offered one rouble and for sick ones five roubles.

Forty Tarbagans were kept for observation in the cages; when we had satisfied ourselves that they were perfectly healthy they were released and others put in their place. In this way about 80 Tarbagans came under observation. During the week we spent at Borsja, no sick Tarbagans were brought in and none of those under observation showed any sign of disease. On the day of our arrival at Borsja we performed post-mortems on five Tarbagans and found all of them healthy.

Prof. Zabolotny, Dr Ch'en and I drove in carts and also walked over the whole neighbourhood, visiting particularly the places where sick and dead animals were reported to have been found in large numbers.

On July 24th we drove to Tschintansk, a village south-west of Borsja. There were plenty of Tarbagans on the way and we stopped and examined the country at different stages of the journey, but could find no trace of sick animals.

The driver of our *drosky* had been in these parts for the last 27 years. He informed us that as early as 1884 Tarbagans had died from an epidemic in this neighbourhood and that one day a Russian doctor and his dresser, stationed at Akscha, performed a post-mortem examination on a hunter who had died under suspicious circumstances. They were both taken ill, and two days after performing the post-mortem, died in this man's *drosky* whilst trying to get back to Akscha. He remembered that both were exceedingly short of breath. Unfortunately for the veracity of this account, our *isvostchick* was very fond of vodka as we experienced after leaving Tschintansk—he driving very recklessly, and eventually dashing up a high bank and upsetting the carriage on the top of us! From Tschintansk we went to Arabulak—a “village” consisting of one solitary post-house and looked after by a Cossack family. Here we stopped overnight. The country between Tschintansk and Arabulak as well as the high hills surrounding the latter place abounded in Tarbagans. Our party divided up and we inspected hundreds of holes but found no trace of sick or dead animals—not even Tarbagau remains being seen.

On the following morning we got up at 4 a.m. to see if the Tarbagan came out from its hole at dawn seeking food as some believe to be the case. But during the next six hours we did not see more than five Tarbagans. We then returned to Borsja.

In the afternoon (July 25th) we went out all around the neighbourhood of Borsja, and although we inspected many holes, no trace of a sick animal was found. On July 27th a dead Tarbagan was found four versts away. We judged the animal to have been dead some 48 hours. The lymphatic glands were dark in colour but not enlarged, whilst the spleen was double its normal size. Full bacteriological examination, however, did not reveal any *Bacillus pestis*.

On July 29th we all returned to Manchouli, it having been decided that the Chinese Expedition should pursue further investigations in Mongolia. While at Borsja we performed experiments to try and ascertain if cannibalism was common among Tarbagans. In one of these an animal was starved; after some days a dead animal was introduced into the same cage but at the end of five days the carcass had not been mutilated. I wish to take this opportunity of expressing my indebtedness to Prof. Zabolotny for his unfailing courtesy on all occasions when I had the pleasure of being associated with him.

VI. MANCHOULI. July 29th–August 3rd, 1911.

After our return from Borsja to Manchouli Dr Adolphe, surgeon to the Railway, informed me on August 1st that three dead bodies had been found eight days previously some seven versts from the town. Having obtained further particulars from the President of the Russian Municipal Council, accompanied by Dr Adolphe I visited the spot. The way was past the old burial ground where six Russians and 400 Chinese dead of the Plague epidemic during the last quarter of 1910 had been buried in a long U-shaped trench six feet underground. After prolonged search we came across some human remains about two miles from the burial ground. The remains consisted of one skull, the bones of the thorax and a few bones of the limbs; in addition rags and clothes were scattered about. There were also planks—the remains of coffins—and three holes where these coffins had evidently been buried. There were also inscriptions in Chinese, from which we learnt that the men were Chinese and had died on the 14th, 15th and 21st of the eleventh Moon (about the end of November)—this corresponding to the period when Plague was prevalent at Manchouli. On the following day, accompanied by the Chinese Prefect, I returned to the place and after a further search found some more bones but no skulls. We collected the remains together, burnt them, and buried the ashes. Slides and cultures taken from the flesh still adhering to the skulls revealed no *Bacillus pestis*.

VII. WORK IN MONGOLIA. August 4th-13th, 1911.

On August 4th we left Manchouli for Mongolia. Our party and equipment consisted of:—

- Three medical men—Drs Ch'en, Tsang and myself;
- One Interpreter who spoke Russian, Mongol and Chinese;
- One Sergeant and six mounted Policemen (Chinese);
- One Finn Tarbagan hunter; this was the hunter who caught, in the previous April, the 12 Tarbagans for the International Plague Conference;
- Two carriages;
- Three carts for tents, baggage, provisions, etc.;
- Nineteen horses and ponies.

Our laboratory equipment consisted of two microscopes, *media* ready for use, traps and snares for Tarbagans, cages, apparatus for experiments with fleas, etc. The weather was good all day and in the evening we encamped at Tarbagan Ta Hu, 30 *li*¹ south-west of Manchouli. The water in a lake close by was briny and we had to depend on the stock brought with us. Here swarms of mosquitoes were encountered.

Mosquitoes.

Tarbagan Ta Hu, 30 *li* south-west of Manchouli, August 4th, 1911, near a small lake of briny water.

Mosquitoes present in enormous numbers, the air being black with them. The gauze covers around our hats were of no avail as the insects covered them making it impossible to see, and owing to their having long proboscides they were able to attack the face. I collected some hundreds of mosquitoes and found them to be:—

- (1) *Culex* principally, and
- (2) *Anopheles maculipennis*.

(These were afterwards confirmed by Professor Nuttall of Cambridge.)

Later I found that the Mongols complained of fever, and since *Anopheles* was present in this neighbourhood, one presumes the disease to be malaria. Some Mongols seen during our expedition were suffering from what was undoubtedly malaria clinically, and the disease responded to treatment with quinine.

¹ 1 *li* = $\frac{1}{3}$ mile.

CHARBADA.

On the following day, August 5th, we reached Charbada—a village 63 *li* south-west of Manchouli. The country *en route* showed nothing but long grass and Tarbagan mounds. The village is made up of a few huts occupied by about 20 Mongol families numbering under 100 souls. As soon as the grass has been cleared by their large droves of horses, cattle, and sheep, they move their dwellings to other places. (For our observations on the Mongols, see p. 259.)

From enquiries made among these Mongols, I learnt that there had never been any outbreak of disease resembling human plague in their midst. Nor could I obtain any word of disease among the Tarbagans: the country in which they had lived for many years past abounds in Tarbagans, yet they had never noticed the animals dying.

We ourselves laid traps and caught several Tarbagans, but none of them showed any signs of disease. We scoured the neighbourhood for remains of the animal, *e.g.* bones, skulls, carcasses, but could find none. Leaving Charbada we made our way backwards along the banks of the River Kerulen, and arrived at Kulun See—a large shallow lake of semi-alkaline water. On the way we found many good camping grounds where there was fresh water. At many such places we found collections of Tarbagan skeletons, each numbering from 50 to 80 sets of bones, lying in heaps—evidently the remains of animals which had been skinned by the hunters who had afterwards thrown away the carcasses. At Kulun See we camped with another colony of Mongols. Here again we could get no history pointing to Plague either among the Mongols or among the Tarbagans. In the country around, although plenty of Tarbagan holes were met with, few of the animals were seen, and our traps seldom caught any. The country had been used for pasturage by the Mongols and large stretches had been fired. From this it would seem probable that, when food becomes scarce in one region, the Tarbagans migrate to regions where food is plentiful.

From Kulun See we proposed to go to Abagaitui (Siberia) by way of Dalai Nor¹, but our guide informed us this was impossible as it would mean crossing mountains where there was no water. We, therefore, returned to Manchouli, reaching there on August 31st.

¹ Dalai Nor is a village 28 versts east of Manchouli and contains a coal mine supplying coal to the Railway.

The Mongols.

(Plate VII, fig. 4.)

At Charbada and Kulun See we camped with Mongols and thus had opportunities of studying them and their habits. The Mongols at Charbada were apparently quite wealthy though they lived in a most primitive way.

The huts are round and domelike measuring about 15-20 ft. in diameter and consist of a series of moveable wooden frameworks $4\frac{1}{2} \times 2$ ft. in size, oblong in shape, and covered by felt matting made of camel's hair or sheep-skins. On the floor is laid felt matting on which the inhabitants sleep. The furniture consists of a few wooden chests where clothes and money are kept, a Buddhist shrine, a cooking pan and stove, and a box containing dried cattle-dung which is used as fuel for cooking and heating purposes. The same pair of tongs used for the dung is employed in their cooking operations.

The Mongols drink a large quantity of cow's and goat's milk and they eat, besides mutton, a good deal of Tarbagan flesh which is only half roasted before the primitive fire. There were some children in the camp at Charbada; these, and likewise the children seen at Kulun See, were clothed in rags and were indescribably dirty. All the people looked healthy, however, in spite of their peculiar mode of living. The Mongols are a simple contented people. They informed me that if more than one son were born in a family the other sons must become *lamas* (priests). Hence the population has decreased enormously during the last 200 years. While the men tend their flocks, the women look after the homes. A large percentage of those whom we encountered had passed their 60th year, and one old man was at least 80. Doubtless the open air existence with the dry atmosphere preserves these people remarkably well in spite of the trying weather, but at an early age their faces show signs of toil. They were very hospitable and willingly supplied us with milk for which they declined to be paid. Their carts appeared crude and rickety, having no metal rims around the wheels, or metal work at the joints: yet they seemed to answer the purpose well.

VIII. THE TARBAGAN OR MARMOT. (*Arctomys bobac*, Schreb.)

(Plates VIII-X, figs. 5-9.)

The Russians call this Marmot "Szuriok" and the Chinese "Han Ta"; but the name most familiar is the one adopted by the members of the International Plague Conference, namely "Tarbagan"—a term derived from the Mongols.

The Marmots belong to the group of simple-toothed Rodents which include Squirrels, Rats, Mice and Porcupines.

The particular species of Marmot found in Manchuria and Mongolia is identified as *Arctomys bobac*, Schreb. (or the true Marmot).

The following are some of its distinguishing features:—

1. The body is stout and the limbs are short.
2. The tail is bushy and comparatively short, being about one half the length of the body.
3. The head is wide and short and there are no cheek pouches.
4. The eyes are large and full.
5. The ears are small and more or less rounded.
6. Of the five toes, the thumb is rudimentary, being supplied with a flat nail, whilst the claws of the remaining four are long and exceedingly sharp.
7. The rows of molar teeth are placed nearly parallel to each other, both in the upper and lower jaws.
8. The fur is of moderate length and of a fine texture. The general colour tends to change at different seasons of the year, varying from a light greyish brown in the spring to a reddish brown hue in the late autumn. On the back and around the eyes the fur is darker in colour.

9. The length of the adult animal exclusive of tail varies from 15 to 18 inches (37 to 45 cm.).

10. The weight of the adult animal varies from 9 to 12 lbs. (4100 to 5400 grammes), being greater as the hibernating season approaches.

After returning to Manchouli from Mongolia, a series of morning and evening temperatures were taken on these animals by us (see Appendix I, pp. 278, 279). The temperature taken *per rectum* presented wide variations in different animals and in the same animal at different times—even in apparently perfect health. Thus, in some it was found to be 95° F., in others 96° F., in others again 97° F., and so on until in one animal 107.6° F. was registered. This last case was particularly interesting in that this

temperature was obtained soon after capture. It died, however, 20 minutes after the temperature was taken and 61 minutes after capture. At the post-mortem nothing abnormal was found. In one of the animals the temperature varied from 98.5° F. to 104.8° F. within the space of three days.

Distribution of the Marmot.

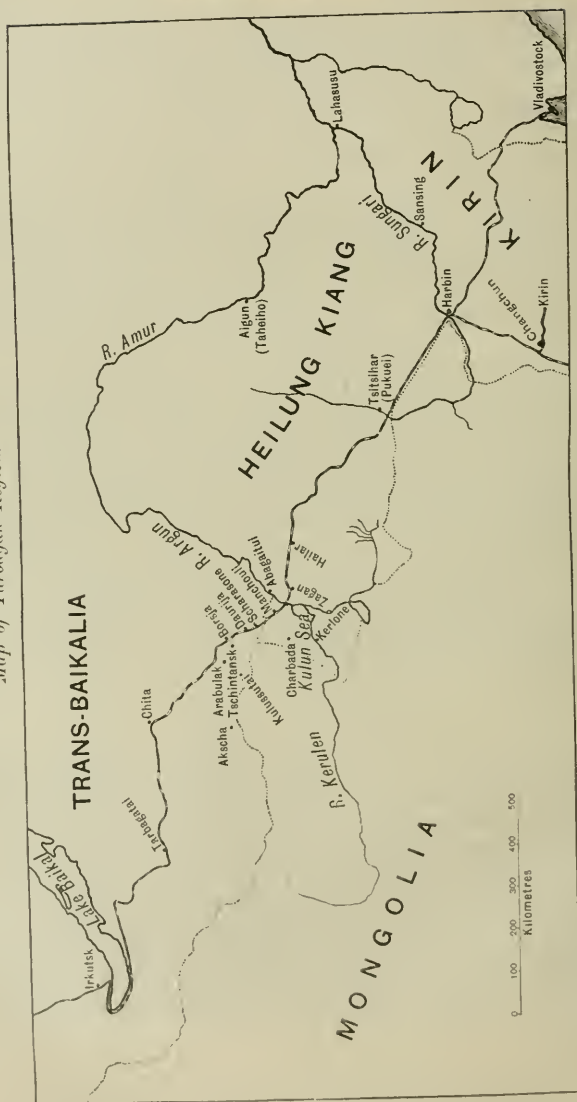
The Marmot inhabits a wide range but is confined to the Northern hemisphere. In North America the common species is the Woodchuck, the distribution of which is from the Carolinas northward to Hudson's Bay, and westward from the Atlantic Coast to Missouri, Iowa, and Minnesota; but other species are met with in the Rocky Mountains and in the north-western parts of America, even as far as the Arctic Regions.

In the Old World, the best known species are the Bobac (*Arctomys bobac*, Schreb.) and the Alpine Marmot (*Arctomys marmota*). The region of the former extends from the south of Poland and Galicia over the steppes of Southern Russia and the bare regions of Siberia to Northern Mongolia, North-West Manchuria, the Amur Regions, and on to Kamstchatka, whilst it is found in elevated regions as far south as Cashmere, Thibet, and the Himalayas, but the southern limits have not been defined accurately. The Alpine Marmot is confined to the higher regions of the Alps, Pyrenees and the Carpathians. A small species—*Spermophilus citellus*, Linn.—is found in abundance in South Manchuria, especially around the neighbourhood of Mukden. This species bears little resemblance to *Arctomys bobac*.

Habits of the Tarbagan.

In the regions where the Tarbagan abounds, it can easily be seen either running about on its four legs or standing on its mound near the entrance to its burrow. It can be made out without difficulty in the distance as the immediate neighbourhood of the entrance to its burrow and part of the mound is devoid of grass, due no doubt to its constant presence there and to its having eaten away the grass. One or more animals may be seen resting on one mound, sometimes on all four limbs, but more often on the hind legs, the fore paws being raised with the palms turned forwards. They like the sunshine and seem to enjoy nature, uttering a noise, when at ease, similar to "pi ah, pi ah"; this has been likened to "pu p'a, pu p'a," which in Chinese means "no

Map of Tarbagan Region.



harm, no harm." The ears are very sensitive to touch but hearing is not as acute as in hares, and hence they make more use of their eyes for the detection of any possible foe. On the approach of a stranger they let fall the front paws, and immediately retire into the burrows, to come out again as soon as they feel the danger is past. When running a considerable distance their action is like that of the rabbit. When frightened they utter a cry similar to a child's "eh eh!" The Tarbagan in captivity is a very fierce animal, using his front teeth freely and biting deeply those who come in his way. With his sharp claws he scratches effectively when carelessly handled. For dealing with those we had in cages, strong forceps of considerable length had to be made, each blade curved so as to give a firm grip on the neck or body (see Pl. XV, fig. 19). When placed in a wooden cage lined with parallel bars of soft iron, $\frac{1}{2}$ inch in diameter, the animal escaped in a very short time by bending the iron rods with his strong jaw and by biting the wood of the cage with his sharp teeth. An adult person was unable to bend such a rod.

Habitat.

The Tarbagan regions of North Manchuria and Siberia are well seen from the windows of the Trans-Siberian trains between Hailar and Manchouli, extending for a distance of 650 versts. The characteristic mounds are present everywhere, especially to the north of the line, and not uncommonly the animals are visible sitting outside the entrances to their burrows.

The holes are sometimes single, sometimes multiple, and on one mound as many as eleven have been counted by us. When occupied, the tops of the mounds are usually bare, whereas grass grows abundantly around the openings of deserted burrows. Hunters informed me that in winter when grass is absent, and the animals are hidden inside their burrows, they detect them by the peculiar smell given forth. I myself tried to detect the odour, and was satisfied that it was characteristic. The presence of innumerable mounds, or "bootans" as the Russians call them, is very distinctive of the Tarbagan country, where the land presents a series of undulations, over some of which the grass grows to an unusual length (Pls. X-XIV, figs. 10-18). These mounds have been made from the earth thrown up by generations of Tarbagans in the course of their digging operations. Where the mountains are of rocky formation, as at Arabaluk in Siberia, large pieces of stone, some weighing as much as two pounds, were seen lying at the entrance of the burrows; these

had evidently been dug up by the animals whilst making their burrows, and showed the great strength of their paws. In other parts, however, the land is more sandy in nature, and beyond their raised appearance they present no great variations. The entrance to the burrow is funnel-shaped, and ranges from $1\frac{1}{4}$ to $2\frac{1}{2}$ feet in diameter. At the entrance twigs and seeds are often found, probably the remains of food, and also hard faecal matter. The tunnels vary from $\frac{1}{2}$ to $1\frac{1}{4}$ feet in diameter becoming narrower as they are traced inwards. When two holes are, say, 12 feet apart, a straight connecting path may be seen where grass has not grown, thus showing that frequent communication takes place between the animals. From the entrance, a sloping passage 5 to $6\frac{3}{4}$ feet (150 to 200 cm.) long leads to a horizontal, more or less zigzag tunnel, the depth of which is $3\frac{3}{4}$ to $5\frac{1}{2}$ feet (110 to 180 cm.) from the surface. Along this underground passage there are specially widened spaces, at some of which faecal matter is deposited, whilst at others there are stored dry grass and twigs—apparently the living quarters of the animal. Very often, when other entrances are traced, they are found to lead to a common passage, so that at places a series of subterranean tunnels extending for hundreds of yards may be said to exist. It is interesting to note that in the course of our digging operations the skulls and bones of Tarbagans were found in the passages, showing that in past times Tarbagans had died in their subterranean homes. Considering that the ground in North Manchuria and Mongolia is frozen in winter to over six feet deep, the Tarbagan may be said to pass through his long hibernating period well within the freezing zone, as the burrows seldom reach a depth of 5 feet. Some of these burrows end blindly after a distance of 4 feet (120 cm.), but others communicate freely. This was instanced by the fact that traps laid for days outside one opening did not catch any animal, and stones placed by us to block an entrance did not affect his peregrinations (Pls. XIII–XIV, figs. 16–18).

Regarding breeding, there is reason to believe that the Tarbagan is not as prolific as the rabbit—two or three being the average number of young ones born each season. When the young are old enough to look after themselves, the mother leaves them in the old burrow and digs a new one for herself. Many half completed burrows were met with in the summer of 1911 during the course of our journey. The new burrows in some cases led to old "earths."

Hibernation.

The Tarbagan hibernates from October to April; at the end of September 1911 the weather was becoming cold at Manchouli and Dr Ch'en noticed that some of the animals in the cages began to curl up preparatory to hibernation. In winter the cold is very severe, the thermometer marking -30° to -40° C. Indeed, Marmots seem to be the most thoroughly hibernating of all mammals, since their sleep is apparently unbroken, and they lay up only a small store of winter food, consisting of grass, roots and the seeds of plants. Whilst in this stage they are utterly helpless. In March, 1911, before the cold season had passed away, twenty-four Tarbagans were dug out from their several burrows in the neighbourhood of Manchouli. They were found lying in the nests of soft herbage far away from the openings of the burrows. For some time, however, after their retirement they continue active within their domicile and feed upon the food which they have gathered during the summer; and as a preparation for their winter sleep, they become exceedingly fat during the autumn. With the return of spring comes renewed activity on the part of the animals, and they venture into the open. Hence the hunters choose the months of April, May and June for hunting them. July and August is the breeding season, and the Chinese authorities forbid trapping during these months. After August they are again hunted until the approach of cold weather—about the end of September.

Method of catching the Tarbagan.

The method generally adopted by the Cossack and Chinese hunters for catching Tarbagans is simple in the extreme. A piece of medium-sized iron wire, $2\frac{1}{2}$ feet in length, has one end twisted in the form of a running loop, whilst the other end is wound firmly round a rough wooden peg $\frac{3}{4}$ foot long (Pl. XV, fig. 19). The peg is driven into the ground immediately above or on the side of the entrance to the burrow, and the loop is arranged so that it fits exactly into the opening. When the animal comes out, the head and probably also one of the front paws are caught in the loop. The more it struggles the tighter the snare becomes, and in this state the hunter finds the animal when he returns from his rounds. Sometimes, double loops are attached to a single peg in order to obtain a more secure hold of the animal. The cost of the complete snare is only two copper cents (about one half-penny). In our

own experience the snare proved to be the most successful means of catching the Tarbagan. The hunters informed me that on lucky days they could catch from five to six animals, which meant an earning of 4-5 roubles a day (counting the price of each skin at 80 kopecks).

Method of killing and skinning.

After capture, the animal is killed by a method called "breaking the neck." The animal is seized by the hind legs, a stick or bar of wood is placed on the back of the neck with one foot at either end of the stick, and then the animal is pulled backwards and upwards. This quickly dispatches the creature, and saves the fur from being soiled with blood. From enquiries made it appears that skinning is not done on the spot, but only when the hunters have gathered again in their common camp—frequently after two or three days' absence. Skinning is performed by incising the two corners of the mouth, separating the skin from the soft parts of the jaws and then pulling the complete skin from before backwards and from within outwards, *i.e.* the skin is turned inside out. As previously noted, when travelling in Mongolia, isolated heaps of Tarbagan bones were often seen—evidently the remains of carcasses left after the animals had been skinned. The raw skins or "pelts" are dried, collected together, and put aside until the end of the hunting season, when they are brought by the hunters to the markets of Borsja (Siberia), Manchouli, and Hailar¹ (North-West Manchuria), and sold to dealers—usually Russian Jews.

The Tarbagan in commerce.

The trade in fur has been fully dealt with already (see pp. 251, 252). Besides using the fur, the Siberian settlers and Mongols eat the flesh of the Tarbagan (see p. 259). When at Charbada the members of our party partook of the flesh and found it tender and the taste distinctly good, comparing favourably with the flesh of the rabbit. Considerable quantities of the flesh are salted and exported to European Russia.

In addition, the fat, which is plentiful under the skin of the animal, is turned into a valuable kind of grease much used by Russian peasants for preserving leather; they also apply it for the healing of bruises.

¹ Borsja is 121 versts west of Manchouli. Hailar is 174 versts east of Manchouli.

The Tarbagan in captivity.

Cages (Pl. XV, fig. 20). For keeping the animals we used both single and double cages made of thin iron plates. These had doors in front and behind so as to facilitate cleaning and the transference of the animals from one place to another. The sides were perforated with a few round holes for ventilation. Rods and parallel slits were found impracticable, as the animals easily damaged them and sometimes escaped. For experimental purposes single cages were made with a glass front to allow of observation.

We originally took with us from Harbin a few cages made of wood, one inch thick, and provided in front with soft iron bars half-inch in diameter; but the animals quickly destroyed these, gnawing away the wood and bending the bars.

In Mongolia and Manchouli it was found, as indicated elsewhere in this Report (Appendix I, pp. 278, 279), that Tarbagans often died when kept in close captivity. In March 1913, *i.e.* the end of winter, 13 animals were dug up while still hibernating and placed in cages. Within ten days two had died and before five weeks had passed, four more succumbed. Two Tarbagans presented in 1911 to the Zoological Gardens in Peking were kept on earth in a spacious pen; after a year they were still alive and healthy. We propose therefore to adopt a similar plan for keeping these animals in future.

For food, hay, cabbage, carrots, and ground nuts have been found satisfactory; a pan of water in the cage does not seem necessary.

Forceps for handling the Tarbagan.

It was some time before we found the most convenient and suitable type of forceps to use in handling Tarbagans. The pair of forceps eventually employed (Pl. XV, fig. 19) is made of stout wrought iron, 20 to 22 inches long, and thickest at the handle. The blades used at the end for gripping the animal are curved to form an oval ($4\frac{1}{2}'' \times 3\frac{1}{4}''$)—this being large enough to take hold of the neck or body without injury. Distal to the oval the blades are prolonged parallel to each other for a distance of half-inch and are there rounded off.

Parasites of the Tarbagan.

In its natural state the Tarbagan harbours two kinds of blood-sucking arthropods, namely the flea and the tick. The fleas were more numerous on the animal when freshly caught than after it had been in captivity

for some time. Most of the fleas were caught in the groins, but often they were distributed over the whole body. The ticks were usually attached to the eyelids, but on one occasion we found two ticks on the abdomen and none on the eyelids. The number of fleas per animal varied greatly even just after capture. For example, we caught 94 fleas on a Tarbagan at Charbada; the fleas on Tarbagan No. XVIII (see page 279) were noted as numerous; on Tarbagan XXIII, three days after capture, no fleas were found but only four small ticks and one large tick; on Tarbagan XXVIII (see page 279) two fleas and two ticks were found. All the fleas were of the same species, namely *Ceratophyllus silantieri*, Wagner 1898. I append a short description taken from those we collected.

Fleas.

Ceratophyllus silantieri (Pl. XVI, fig. 22). An eyed, single-combed flea of large size. Of six males, the shortest was 1.97 mm. long, the longest 2.34 mm., whilst the average length was 2.2 mm.; of 13 females the shortest was 1.48 mm., the longest 3.07 mm., whilst the average length was 2.82 mm. The eye is small and the antennae are well developed; the base of the antenna is situated at some distance directly above the eye, and the antenna when lying in the groove is directed downwards and backwards. There are three bristles, one in front of the other, anterior to the eye. The maxillary palps and mouth parts (epi-pharynx, mandible, labium) are long and well developed. Springing from the posterior border of the first thoracic segment is a comb consisting of nine bristles on either side. The mesosternite has a vertical thickening (bar). The inner aspect of the femur is studded with numerous hairs. The posterior border of the hind tibia presents six pairs of bristles. The last joint of the tarsus has six bristles on either side and the claws are fairly long. There are nine bristles inserted on either side near the posterior border of the tergal plates. The antepygideal bristles are three in number on each side.

Biting experiments with Fleas and Ticks obtained from the Tarbagans.

The insects were removed from the Tarbagans directly the animals were caught and were placed in test tubes.

The following experiments were carried out by us when in Mongolia and later on our return to Manchouli. As will be readily understood, the conditions did not permit of detailed and prolonged experimentation

and this record is given merely as a preliminary communication on the subject.

Experiment 1. When at Charbada, some six fleas in a test tube, immediately after their removal from the Tarbagan, were given the opportunity of biting one of us. Even after several minutes none of them had bitten.

Experiment 2. August 10th, 1911. *Ceratophyllus silantievi*, 1 ♂. Starved for three days. Then given facilities for biting the arm of one of the party. The insect moved about for some minutes before biting. It chose a spot in a fissure of the skin and plunged its proboscis deep in. While sucking its body was tilted upwards and the insect was seen to increase in bulk. It remained thus for eight minutes and then, having withdrawn the proboscis, fell on its side. Afterwards it began moving around less actively than before.

The person bitten experienced no pain and very little sensation both when the flea pierced the skin and during the time the insect was sucking. After the flea had withdrawn its proboscis, the site of the puncture was barely visible to the naked eye and with the aid of a hand lens a very slight escape of blood was seen to have taken place from the puncture. There was no after swelling or irritation.

Experiment 3. August 14th, 1911. *Ceratophyllus silantievi*, 1 ♂, 1 ♀, having been starved for some days, were placed on the arm of the police sergeant. Both behaved in a similar manner to the flea used in Experiment 2. After sucking for 10 minutes and before they had withdrawn their proboscides, the experiment was accidentally disturbed and the fleas fell to the ground. In this case also no after irritation occurred.

Experiment 4. August 14th, 1911. *Ceratophyllus silantievi*, 1 ♂, having been starved for four days, was allowed to bite the arm of one of the servants. The insect moved around for about five minutes and then, choosing a fissure of the skin, inserted its proboscis. At first the body of the flea remained in the horizontal position while the distal portion of the proboscis was inserted. Gradually the proboscis was buried completely and by that time the flea's body had assumed the more or less vertical position. As in the preceding experiments, there was an almost complete absence of after-effects.

The Ticks (a species of *Rhipicephalus*¹) were collected in test tubes and similar experiments to those with the fleas were carried out. In no case did a tick bite the arm of a human being, although the tick used in one experiment had been starved for eight days.

¹ ? *R. haemaphysaloides*, nymphs.

IX. SUSCEPTIBILITY OF THE TARBAGAN TO ANTHRAX.

Anthrax is a disease very common in Siberia where it attacks both human beings and horses, it being known in the latter as the "Siberian Pestilence." After our return to Manchouli on August 14th we determined to find out whether the Tarbagan was susceptible to this disease as no experiment of the kind had been performed before.

Experiment I. Tarbagan XIV. On the evening of August 20th, 10 c.c. of a 24 hours' old bouillon culture of *Bacillus anthracis* were inoculated into the loose subcutaneous tissue of the back of a healthy adult Tarbagan. The animal became very ill, and on the morning of August 23rd was found curled up and lying on its side dead.

The temperature recorded was as follows:—

August 18th,	E.	98.6° F.
19th,	E.	101.7 "
20th,	M.	99.8 "
	E.	98.4 "

10 c.c. bouillon culture of *B. anthracis* injected.

21st,	M.	100.4° F.
	E.	102.2 "
22nd,	M.	100.8 "
	E.	—

Post-mortem Findings.

Slight swelling and marked induration around the point of inoculation. Superficial veins dilated. General venous engorgement. No petechial haemorrhages.

Lungs: very slight congestion.

Heart: coronary vessels dilated; right side of heart dilated.

Liver: enlarged and congested.

Spleen: markedly enlarged and congested.

Microscopically: *Bacillus anthracis* found in the heart blood, blood from superficial veins, peritoneal fluid, pericardial fluid, scrapings from the spleen and liver. No particular variation in the distribution of the bacilli was found. The organism was obtained in pure culture from the heart's blood.

Experiment II. Tarbagan XVII. On the evening of August 20th, 5 c.c. of a 24 hours' old bouillon culture of *Bacillus anthracis* was inoculated into the loose subcutaneous tissue of the back of a healthy adult Tarbagan. The animal became very ill and on the morning of August 23rd was found curled up and lying on its side dead.

The following is the temperature recorded :—

August 19th,	E.	98.2° F.
20th,	M.	96.8 „
	E.	98.2 „

5 c.c. bouillon culture of *B. anthracis* injected.

21st,	M.	100.8° F.
	E.	103.4 „
22nd,	M.	100 „
	E.	—

Post-mortem Findings.

Marked swelling and induration around the point of inoculation. Glands in neck and axilla a little enlarged and showing petechial haemorrhages: femoral glands markedly enlarged. Other appearances, the same as in Tarbagan XIV, Experiment I.

These two experiments prove, therefore, that the Tarbagan is susceptible to Anthrax and the post-mortem findings are similar to those seen in other susceptible rodents.

X. INVESTIGATIONS INTO REPORTED OUTBREAKS OF PLAGUE AT PUK'UEI.

When at Manchouli, it was reported in the local papers that several fatal cases of Plague had occurred at Puk'uei (Tsitsihar) in the quarter allotted to the *maisons publiques*. These reports gave apparently substantial details regarding such symptoms as headache, fever, coughing of blood, etc.—all pointing to an outbreak of pneumonic plague. I determined to visit Puk'uei without delay to enquire into the matter. I arrived there on August 26th. On investigation I found that two or three servants attached to the houses had recently suffered and died from *acute enteritis*, which could be traced to the raw fruit and iced drinks of which they had partaken freely during the prevailing hot weather. There was nothing to indicate an epidemic, and having satisfied myself fully as to these points, I left for Harbin on August 28.

It may be added here that reports of a similar kind had been circulated in Changbun, Dalny and other places. All these on investigation proved groundless.

Simultaneously with the report of Plague in Puk'uei, there came news that four deaths from bubonic plague had occurred among Russians

at Scharasone; these cases were confirmed later on, as well as a fifth case occurring in the same village¹.

Susceptibility of the Tarbagan to Bacillus pestis.

When at Manchouli, August 14th to 25th, we proposed performing some experiments on the susceptibility of the Tarbagan to Plague. Unfortunately our cultures of *Bacillus pestis* proved to be of an avirulent strain. Owing to difficulty of transit we were unable to obtain other strains. Hence the experiments could not be carried out.

That the Tarbagan is susceptible to *Bacillus pestis* has been shown by Strong and Teague, Zabolotny, Dujardin, Beaumetz and Mosny.

XI. EVIDENCE ASSOCIATING THE TARBAGAN WITH PLAGUE AND CONCLUSIONS THEREFROM.

The Tarbagan has been said to suffer from a chronic form of plague not unlike the form seen in rats. From time to time epidemics have been reported as occurring among the Tarbagans causing them to die in thousands. It has been said that hunters easily recognise the sick animals—these often being driven out from their holes to wander about aimlessly until they die. The piteous state of these animals has been graphically described.

If a human being takes plague and has eaten of the flesh of the Tarbagan—a very common food among the Mongols and Cossacks (page 259)—it has been stated forthwith that this is the source of infection. Indeed, whenever a case of human plague occurs in a remote district of the Tarbagan country, it is stated almost invariably that the disease has resulted from the eating of Tarbagan flesh. Take the following excerpt, translated from a paper issued on December 8th, 1912, as an example:—

“News has come from a village Onon-Borzinski, near Chita, to the effect that some Cossacks, three in number, had been stricken with plague and died. It appears that they had returned at the beginning of October (old style) from Zagan-Olnevski where they had been hunting Tarbagans and had brought with them some frozen Tarbagan flesh. On October 31st one of these Cossacks took ill with symptoms of fever and spitting of blood and died after two or three days' illness. The others followed.”

Comment is needless!

¹ Scharasone is the Russian village 30 miles west of Manchouli where Dr Issaief picked up his Plague Tarbagan in June, 1911 (see p. 254).

Some of the epidemics referred to as occurring among the inhabitants of the Kirghiz settlements (pp. 243—247) were said to have been associated with a disease in camels, and one case of human plague was said to have followed the consumption by the patient of the flesh of a camel stricken with plague.

To quote further examples would be useless and to mention individual authors invidious.

It is my purpose to review the facts which have so far been ascertained bearing upon the relationship of the Tarbagan to the outbreak of plague in human beings. It need scarcely be said that before a diagnosis of plague is established a complete bacteriological examination must be made. It has been definitely established that rats suffer from plague and from them the disease is conveyed to man. The chronic form of plague has been found among the gophers and ground-squirrels (*Citellus beecheyi*) of the Western United States by McCoy. Mice and guinea-pigs are susceptible to artificial infection with *Bacillus pestis*. Strong and Teague, Zabolotny, Dujardin, Beaumetz, and Mosny have shown that the Tarbagan also is susceptible, and Shibayama has demonstrated that *Spermophilus citellus*, the small species of marmot common about Mukden (page 261), is susceptible to plague though not so susceptible as the rat.

Since these Rodents are susceptible to plague and since the disease occurs in nature among rats and ground-squirrels, *a priori* it is conceivable that it occurs among Tarbagans in nature.

Let us examine the evidence available to establish this as a fact. An epidemic among the Tarbagans was reported in July 1911 (page 240), and the Russian and Chinese expeditions at once visited the country where this epidemic was supposed to exist. Not only did the expeditions fail to discover a single diseased Tarbagan, but enquiries made by us directly from the hunters showed that they knew nothing of the alleged epidemic. In the experience of these hunters not only had no epidemic ever occurred among the Tarbagans but they had never even seen sick ones. In Mongolia, the Chinese expedition had similar results nor could any news be obtained of disease, past or present, from the Mongol hunters. In passing, I would emphasise that information was sought by us direct from hunters who were experienced men and who had spent their lives living and hunting in the country where the Tarbagan abounds. Moreover two of the police who accompanied us on our Mongolian expedition were formerly Tarbagan hunters; and the Finn hunter whom we took with us had been hunting regularly for the past five years and could recall no case of Tarbagan disease.

Dr Ch'uan, in his paper at the International Plague Conference, already referred to, stated: "I had several talks with the marmot hunters and enquired whether they knew of the occurrence of any cases of sickness, such as blood spitting or of sudden death during their hunting season on the hills. They replied that they had never known of such cases either on the hills or on their return journey, and that only at Dawoolya, Manchouli or other towns did the plague attack the marmot hunters and others." The enquiries we made on this point from the hunters elicited the same information. We did not hear of any case of plague having occurred amongst them while on the plains. It is well to state here that although the Tarbagan hunters dispersed in pursuit of their business, staying away for one or several weeks, they usually reassembled at the regular camps to skin the animals captured. Moreover, although millions of Tarbagan skins were exported yearly to Europe, and thus handled by hundreds, perhaps thousands of people, from the hunter and the railway porter to the factory labourer, no case had ever been reported of plague infection in human beings during the transit of such skins. It is also very doubtful whether plague occurs as a result of eating plague infected flesh. As bearing upon this question the following may be quoted:—"In many instances during the Manchurian epidemic the patient with pneumonic plague must have swallowed enormous numbers of plague bacilli in the saliva and sputum. Nevertheless, in none of the necropsies performed during the epidemic were evidences of primary intestinal infection present, nor was serious involvement of the intestine encountered. This fact certainly speaks strongly against the evidence of primary intestinal plague in man and would seem to show that even if the intestines are sometimes secondarily involved, this condition in human beings must be also a very rare one." (Strong and Teague.)

Moreover, referring to the disease in camels mentioned above, Mr Shuropof, a veterinary surgeon who investigated the possibility of this animal suffering from plague, concluded that the camel was entirely insusceptible to the disease. He thinks that the observations made in the Kirghiz steppes must be received with doubt and that possibly the organism isolated from the dead camel was the *Bacillus bipolaris plurisepticus*, and not the plague bacillus (*Vratch*, No. 52, December 31st, 1911, quoted in *Lancet*, March 9th, 1912, page 688).

The only definite proof that Tarbagans in nature are affected with plague is obtained from the animal caught by Issaief in June at Scharasone, and examined by Zabolotny (pp. 253, 254).

To conclude that a man whose occupation is that of a Tarbagan hunter and who takes plague has been infected from a Tarbagan is comparable to concluding that a man who sells rice and who develops plague has been infected from the rice. In the latter case it is possible that the rice through the rat flea was the source of infection; but if, without some proof that this were so, the statement was made, such a conclusion would be at once condemned as unjustifiable. From the above it seems to me a pity that responsible authorities and medical men should be so obsessed with the unestablished idea of the great infectivity of the Tarbagan as to place hindrance in the way of transporting by rail live healthy Tarbagans for important scientific research at our headquarters laboratory at Harbin, an experience which I had in March of this year.

Conclusions.

1. Even though the Tarbagan occasionally suffers from Plague, the epizootic is never extensive, and the animal does not play nearly so important a rôle in the spread of Plague as does the Rat. Indeed its direct relationship to human plague may be considered negligible. Moreover, the mode of living and habits of the Tarbagan are very different from those of the Rat: for example, while the Rat is a more or less domestic creature in close contact with man, the Tarbagan is the reverse.

2. From the writings of Russian authorities, it appears that Plague has existed for many years in various parts of Siberia, sometimes in the bubonic form, sometimes in the pneumonic form. These places may be looked upon as endemic foci. In 1910 it is believed that pneumonic Plague appeared in the Russian Ural District long before it made its appearance at Manchouli and developed into that great Manchurian epidemic. During the latter half of 1911 this form of Plague was present in the Kirghiz settlements. In these districts, from October 1911 to February 1912, over 200 cases of Plague occurred. No case of Plague, in man or animal, has occurred in Manchuria since the epidemic of 1911.

3. From this report it is obvious that statements of the occurrence of Plague among men or animals should be believed only when they come from responsible sources—that is, after proper medical and scientific investigations.

XII. RECOMMENDATIONS REGARDING TARBAGAN FUR TRADE.

Writing from Manchouli Station on August 21st, 1911, the following were some of the recommendations I made to the Chinese Government in regard to the Tarbagan Fur Trade:

"1. That as far as this year is concerned, the Order (prohibiting the hunting of Tarbagans) should stand so as to allow the animals a chance of breeding. In a large part of the territory within 100 *li* of Manchouli, the animals were practically exterminated last year, some 2,000,000 having been killed.

"2. That two central stations, say Manchouli and Hailar, should be established, with a medical staff at each place. To these stations the skins should be sent for inspection and disinfection (if necessary). After examination and disinfection (if necessary) a chop¹ should be imprinted on each skin, certifying its healthy state and allowing it to be exported.

"3. That the usual tax of roubles 6.80, should be levied on every hunter for each season. Last year (1910) two seasons were permitted, but I would suggest only one season be permitted in future so as to preserve the animals from extinction.

"4. That whenever permits are issued to the hunters, directions (verbal as well as written) should be given for protection against possible infection, such as keeping the raw skins away from their sleeping quarters and supervising, if not establishing, lodging houses where the hunters may lead a hygienic life."

I further stated:

"In carrying out these recommendations, doubtless a few difficulties would be encountered, but I am sure the best method is to control in a rational manner the Tarbagan trade rather than to abolish it entirely. A very small percentage indeed of Tarbagans catch the disease, and supervision would be quite an easy task.

"The following are some of the advantages of effective control:

"1. The poor people will be profitably occupied and a large and increasing business will be saved from destruction.

"2. Good scientific work will be done by the doctors and hence our knowledge of Plague and of Tarbagan diseases will be increased."

¹ Government mark or stamp.

XIII. OUTBREAK OF PLAGUE ON S.S. *CHEONGSHING*.

At the beginning of June 1912, I received instructions to enquire into some deaths from Plague which were reported to have occurred in Tientsin. I ascertained the facts to be as follows:

The S.S. *Cheongshing* arrived at Tientsin direct from Hongkong on the evening of May 31st, 1912.

The first purser's wife, aet. 30, died on board on May 29th, while the boat was at sea.

On June 2nd the first purser, aet. 43, complained of shortness of breath and faintness. He was diagnosed as suffering from pneumonia, and was admitted on June 3rd to a mission hospital in Tientsin; the patient died a few hours after admission. On June 4th the second purser, aet. 36, complained of tightness of the chest and cough with blood-stained sputum. He was admitted to a Government hospital with the diagnosis of pneumonia. He died early next day (June 5th). Lung puncture, performed after the death of the patient, revealed *Bacillus pestis*—this being confirmed afterwards by complete bacteriological examination.

The Port Health Officer did not diagnose and hence did not certify plague, and allowed the steamer to leave Tientsin on June 5th bound for Chefoo. Off Chefoo the vessel was quarantined on account of a wire from the Chinese authorities and was not permitted to approach the town. On June 8th the vessel sailed for Shanghai.

At Woosung, ten miles from Shanghai, the vessel flew distress signals as more cases of plague had occurred; one fireman and one seaman had died on June 14th. The vessel was quarantined and the crew and passengers were isolated. No further cases occurred and the steamer reached Hongkong in safety.

Later I ascertained that the female patient had lived in a house in Hongkong where two deaths from plague had occurred just before coming on board.

In connection with these cases of pneumonic plague it is interesting to observe that the wife of the first purser was supposed to be suffering from pulmonary tuberculosis. To ease the cough the second purser lent the patient his opium pipe to smoke, afterwards using it himself. In all probability the husband of the patient smoked from the same pipe, as in such cases it is customary for another person to prepare it for use.

Whether the pipe was the direct means of conveying the infection is problematical as the trio had been in close communication in using the small ship's cabin: it is certain that the usual spread of pneumonic plague is by the direct inhalation of the micro-organism.

APPENDIX I.

Rectal Temperature of the Tarbagan.

The series of temperatures given in the accompanying table were taken at Manchouli after our return from Mongolia.

The animals were kept in cages. An animal was removed from the cage by means of the special forceps and was held resting on the ground by one of us while the other took the temperature (see Pl. XVI, fig. 21). The morning temperature was taken about 9 a.m. and the evening temperature between 3 and 5 p.m.

Often after capture by the snare the animal died, no obvious cause being found at post-mortem examination. A larger percentage of those caught with double snares died than those caught with single snares.

PROTOCOL.

Tarbagan No. XI. Healthy during the whole time the observations were taken.

Tarbagan No. XII. September 9th. Appeared sick, lying quite still and apathetic; does not resent provocation; all limbs paralysed, unable to move when placed on back; no crying; no convulsions. Death. *Post-mortem*: some of the glands in neck congested but not enlarged; spleen enlarged but not congested; liver shows fatty degeneration; gall bladder distended with gas, no bile, adherent under surface of liver; small infarcts in upper lobe of right lung; pericardial sac contains more fluid than normal. Films made from the blood and organs show no micro-organism; no growth obtained in media inoculated with heart blood or from spleen.

Tarbagan No. XIII. August 22nd. Appeared ill in the afternoon. Hind limbs paralysed. Died early on August 23rd. *Post-mortem*: all organs normal. Films and cultures negative.

Tarbagan No. XIV. September 9th. Paralysis of hind legs; resents provocation; utters no cries when brought out of cage; attempts to escape using fore legs only, hind legs being dragged. September 11th. Appears rather better, hind limbs still paralysed. September 13th. Cries when handled. September 18th. *In statu quo*.

Tarbagan No. XVI. Died suddenly on night of September 13th. *Post-mortem* showed cause of death to be a large rupture in the left lobe of the liver. Examination for micro-organisms negative (films only).

Tarbagan No. XVIII. Caught morning of August 19th. Attempted to escape while being brought to camp in sack. 3 p.m. Convulsions while lying on ventral surface; appeared to be biting at cage in attempt to escape. 4.10 p.m. Quite apathetic; temperature 107.6; had convulsions while temperature was being taken; numerous fleas. 4.30 p.m. Gave a few gasps and died. Weight 10 lbs. (English). Length, tip of nose to tip of tail, 25½ inches (64½ cms.).

Post-mortem: nothing abnormal found.

Tarbagan No. XIX. Young animal. August 23rd killed and cooked.

Tarbagan No. XX. Healthy during the whole time the observations were taken. Killed for anatomical observations September 4th. Weight 9 lbs. Length, tip of nose to tip of tail, 24½ inches.

Tarbagan No. XXI. Healthy during the whole time the observations were taken.

Tarbagan No. XXII. Healthy up to August 27th when it showed symptoms similar to those noted above for Tarbagan XII and Tarbagan XV. Died in the afternoon. *Post-mortem:* organs showed similar appearances to those noted under Tarbagan XII. Smears made from the glands and organs showed bacilli which, however, were obviously not *Bacillus pestis*; no growth in media even after six days.

Tarbagan No. XXIII. On August 28th died suddenly without previously showing any signs of illness. *Post-mortem* findings similar to those of Tarbagans XII, XV, XXII. Smears similar to those from Tarbagan XXII. No growth on media even after four days.

Tarbagan No. XXIV. Healthy during the whole time the observations were taken.

Tarbagan No. XXV. Healthy during the whole time the observations were taken.

Tarbagan No. XXVI. Healthy during the whole time the observations were taken. On September 30th the animal curled up through cold.

Tarbagan No. XXVII. In good health up to September 7th, then did not appear to want to feed. Died on September 18th. Very emaciated. *Post-mortem* examination revealed a small rupture in the right lobe of the liver; adherent blood clot was present in the neighbourhood. Other organs apparently normal. Bacteriological examination negative.

Note. An animal (Tarbagan No. XXVIII) caught on August 25th died soon after being brought into camp. There were marks of severe constriction by the wires of the double snare around the neck and at the level of the umbilicus. *Post-mortem* examination showed no abnormal appearances.

Plague in Manchuria

Rectal Temperature in degrees Fahrenheit of 17 Tarbagans.

AUG. 14th—Sept. 13th, 1911.

Tarabagan No. 1		XXI	XXII	XIII	NV	NVI	NVIII	XIX	XX	XXI	XXII	XXIII	XXIV	XXV	XXVI	XXVII	XIV	XVII
Aug. 18	M.	100-8	98-6	...
19	E.	104-8	101-7	98-2
20	E.	95	99-2	104-8	100-2	100-2	...	98-4	99-8	96-8
21	E.	99-8	98-6	101	97-8	98-6	107-6	100-4	98-4	98-2
22	E.	98-7	97-8	100-2	97-8	98-6	D	96-5	100-4a	100-8
23	E.	99	97	98-5	98-8	97-8	...	103-6	102-2	103-4
24	M.	96-4	97-6	95-2	99	96-4	...	K	99-6	102-8	100-8	100
25	E.	99-8	98-6	D	95	98-8	D	D
26	E.	97-6	98-6	98	97-2	98-6	...	98	98-4	99-8	99-4	D	D
27	M.	96-4	98	97	96-2	102	96-2	99	97-6	99-4	101-7	98-2
28	E.	99	99	98-4	97-5	98-4	400	100-1	100-1	101-7	98-2
29	M.	97-5	99	...	98-4	97-5	95-2	96	98-8	101-6	98-6	98-6	98-8	98-8	100-8	100
30	E.	99-2	100-1	...	98-6	97-8	100-6	96-4	100-2	100-4	99-2	98-8	98-8	98-8	100-8	100
Sept. 1	M.	97-2	97-6	99-1	99-1	100	95-2	97-2	102-2	102-2	99-2	96-8	96-8	96-8	100-8	100
	E.	100	101-4	...	99-9	99	97-1	100	95	100-4	98	96	98-5	102
	M.	99	99-6	160	99-2	99-2	97-2	98-2	...	102-6	97-2	96-8	99-6	98-4
	E.	99	99	99-6	99-4	99-4	96-4	96-4	D	100-2	99	95-4	99-6	98-4
	M.	96-8	98-6	102-2	96-4	96-4	96-4	96-4	D	100-2	99	95-4	99-6	98-4
	E.	97	98	100	99	99	97-6	96	...	100-8	97-6	97	97-6	97-7
	M.	97-6	98-4	...	98-8	98-8	98	99-2	...	95-2	100-6	97	97-6	98-8
	E.	101-1	100-1	100	98	98	98-4	97-6	99-4	97-2	99-2	100-1
	M.	99-6	100-1	...	98-8	98-8	99	98-2	...	D	99-4	97-2	98-8	99-8
	E.	97-2	102-4	...	99	99-4	101	97-8	99-4	97-4	102-8	98-6
	M.	97-4	99-2	...	99-2	98-4	97-4	97-4	101	97-8	101	99-4
	E.	98-2	99	...	98	98	96-8	99-2	100-1	97-4	98	98-2
	M.	99	99	...	99-8	99	97-2	98-4	101-7	97-4	98	98-2
	E.	95-7	97-7	...	97-7	97	99	99	101-7	101	101	99-2

2	M.	98.2	99.2	...	98.8	98	98.8	99.4	98.8	98.8	98.6
3	E.	99	97	98.6	97.6	99.2	97.8	99.8	98.4	99	97.4
4	E.	99	98	...	100	100.2	99	99	99	98.4	99
5	E.	96.8	98	...	99.4	100	97.8	100.2	99.4	99	98.2
6	E.	102	99.6	99	98.6	99.8	98.8	98.6	98.6
7	E.	99.2	99.2	...	98.6	97.8	98.6	98.4	98.8	98.6	98.6
8	E.	99.8	97.6	...	101.2	98.8	98.6	98	98.6	98.8	97.8
9	E.	99.8	99	...	100.2	98.8	K	99	98.6	98.8	98.6
10	E.	99.6	99.8	...	99.4	98.6
11	E.	99.6	99	...	99.8	99.2
12	E.	97.8	99.4	...	99.1	99.2
13	E.	100.2	100	...	99.8	98.6
	E.	98.8	97.2	99.2
	E.	100	98.6
	E.	99.6	D
	E.	98.4
	E.	99.6	99.6
	E.	98.8	98.9
	E.	100.2	100
	E.	98	97.6
	E.	99.2	98.6
	E.	99.4	D
Highest temp.	M.	100.2	100.1	100.2	102.2	100	99.6	102.8	101.6	100.6	99.8	99.8	102
Lowest temp.	E.	102	102.4	101.8	100.6	102*	101	100.1	104	102.6	101.7	101	102.8
	M.	96.4	97.2	95.2	97	96.4	95	96	85	95.2	98	95.4	100.1
	E.	95	95.7	98.5	97.6	97	96.8	96	99.4	100.1	97.2	96.8	97.4

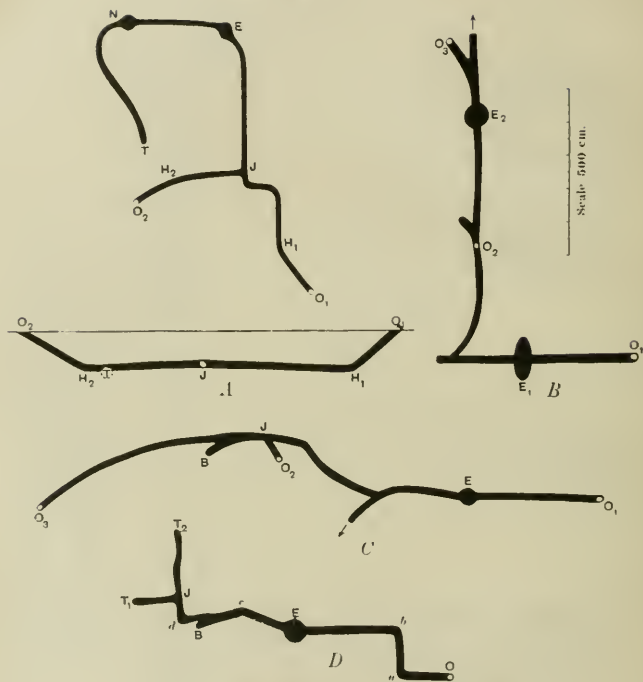
D=Died

* Animal escaped; temp. taken just after recapture.

K=Killed.

a=inoculated with *Bacillus anthracis*.

APPENDIX II.



Tarbagan Burrow A. Near Manchouli. Opened Aug. 19th, 1911.

O_1 , O_2 , entrances to the burrow. J, junction of the tunnels from entrances O_1 , O_2 .
 E, enlargement containing faeces. N, enlargement containing "nest." T, terminus.
 Diameter of entrance O_1 21 cm. Diameter of entrance O_2 28 cm. O_1 to H_1 195 cm.
 O_2 to H_2 239 cm. H_1 to J 452 cm. H_2 to J 355 cm. J to E 276 cm.
 E to N 284 cm. N to T 479 cm. Whole length from O_1 to T 1633 cm.
 Diameter of E 72 cm. Diameter of N 58 cm. Distance from O_1 to O_2 623 cm.
 Depth at J 102 cm. Depth at T 124 cm.

Tarbagan Burrow B. Near Manchouli. Opened Aug. 19th, 1911.

O_1 , O_2 , O_3 , entrances to burrow. E_1 , E_2 , enlargements.
 Diameter of entrance O_1 23 cm. Distance from O_1 to horizontal 304 cm. O_1 to
 E_1 308 cm. Size of E_1 140 by 50 cm.

Tarbagan Burrow C. Near Manchouli. Opened Aug. 22nd, 1911.

O₁, O₂, O₃, entrances to burrow. E, enlargement. J, junction of tunnel from entrance O₂. B, blind end. From entrance O₁ the tunnel sloped downwards to E, a distance of 304 cm.
 E to J 725 cm. J to B 177 cm. J to O₃ 780 cm. Deepest part of the burrow 110 cm.
 The blind end B connected with the surface. From O₂ to O₃ the ground was very stony.

Tarbagan Burrow D. Near Manchouli. Opened in March 1911.

Measurements taken in Aug. 1911.

This was one of the Burrows opened by the Finn hunter in March 1911 to obtain Tarbagans for the International Plague Conference. When opened the ground was frozen and the Tarbagans were hibernating.

O, entrance to the burrow. E, enlargement. B, blind end. T₁, T₂, Tarbagans found here by the hunter.

O to a 166 cm. a to b 142 cm. b to c 385 cm. c to B 142 cm. B to d 72 cm. d to J 72 cm. J to T₁ 129 cm. J to T₂ 213 cm.

APPENDIX III.

Translation of the Chinese Order prohibiting the hunting
 of Tarbagans (April, 1911).

*Prohibition Order regarding Tarbagan Hunting,
 Hsuan Tung, 3rd Moon, 20th Day.*

By The HULUN TAOTAL.

1. Anyone found in possession of traps or other instruments for catching Tarbagans is liable to have these traps or other instruments confiscated and will not be allowed to continue to hunt.
2. Any cart found conveying Tarbagans or Tarbagan skins is liable to confiscation. The driver of the cart and the hunter will be punished.
3. Any tent intended for the use of Tarbagan hunters and any Tarbagan skins found by the Government officials are liable to be burnt and any hunter found disobeying this Order will be punished by 6 months' imprisonment.
4. No Chinese or Russian is allowed to sell Tarbagan skins. Anyone found disobeying this Order will be arrested and handed over to the proper authorities for punishment.
5. Any Chinese disobeying this Order will be tried by the local authorities. Any Russian disobeying this Order will be handed over to the Russian authorities for trial.

The above Orders are made in order to prevent a recurrence of Plague.

Table of temperature observations in Fuchiatien (Harbin).

SERIES A.		Description of building	No. of persons at the time	Approx. size of room, in feet L. B. H.	How heated	Did plague occur here? few or many?	Temp. inside room, (°C.	Temp. outside room, (°C.	Date	Time of day taken
No. of obs.	Variety of dwelling									
1 a	Native Inn	Mud-walled room, old, no windows, native kang on both sides. Ground of room about 1 ft. lower than that outside the door. Earth floor	11	23 19 12	Brick stove with chimney	Not sure	10	-3	2/2/13	12:00 noon
1 b			15				15	-4		9:30 p.m.
2 a	Native Inn	Brick wall outside, mud inside, earth floor, 1 window, kangs on both sides leaving passage in the middle	11	19 16 9	Brick stove with chimney	Not sure	7	-2	do.	1:00 p.m.
2 b			12				10	-4		10:00 p.m.
3 a	Native Inn	Earth floor, paper windows 6, kangs on both sides	12	26 19 7	Brick stove with chimney	?	12	-2	do.	2:30 p.m.
3 b			12				11	-5		11:00 p.m.
4 a	Native Inn	Plank floor, native kangs on both sides, 2 paper windows	14	23 20 8	Open charcoal pan	?	10	-2	do.	12:00 noon
4 b			14				14	-5		4:00 p.m.
5 a	Native Inn	Earth floor ...	12	18½ 16½ 9½	Stove with chimney	?	17	-2	do.	2:00 p.m.
5 b			12				22	-5		10:00 p.m.
SERIES B.										
6 a	Native Inn	Plank floor, kangs on both sides of room, glass windows	22	17 17 7	Open charcoal pan	?	16	-3	3/2/13	2:00 p.m.
6 b			8				22	-10		10:00 p.m.
7 a	Native Inn	Plank floor ...	8	16 19 7	Kangs	?	16	-3	do.	3:00 p.m.
7 b			9				17	-10		11:00 p.m.
8 a	Native Inn	Earth floor, kangs ...	8	19 19 7	Kangs	?	14	-4	do.	3:00 p.m.
9 a	Native Inn	Earth floor, mud and straw wall, 4 paper windows	14	19 19 10	Chimney stove	?	10	-3	do.	1:00 p.m.
9 b			14				11	-7		8:00 p.m.
10 a	Native Inn	Plank floor, 1 window	7	9 19 9	Chimney stove	?	12	-3	do.	3:00 p.m.
10 b			7				15	-7		9:00 p.m.

11 a	Native Inn	Plank floor, kang on both sides	6	23	21	9	Kangs	?	16	-4	do.	4.00 p.m.
11 b			0						16	-8		10.00 p.m.
SERIES C.												
12 a	Native Inn	Earth floor, 4 windows	14	19	19	9	Stove with chim-ney	?	10	-3	4/2/13	3.00 p.m.
12 b			0						13	-7		9.00 p.m.
13 a	Native Inn	Earth floor, 2 windows	8	21	12	9	Stove with chim-ney	?	13	-8	5/2/13	4.00 p.m.
13 b			8						18	-15		8.00 p.m.
14 a	Native Inn	Plank floor, 1 window	5	7	12	12	Open charcoal pan	?	15	-9	do.	5.00 p.m.
14 b			5						18	-17		9.00 p.m.
15 a	Native Inn	Brick floor, 1 paper window	9	21	21	8	Stove with chim-ney	?	21	-8	do.	8.00 p.m.
16 a	Private	Earth floor, kang, 2 paper win-dows	2	19	7	8	Stove with chim-ney	?	16	-15	6/2/13	2.00 p.m.
16 b			2						17	-19		9.00 p.m.
SERIES D.												
17 a	Native Inn	Earth floor, 4 windows, mud wall	12	23	19	7	Stove with chim-ney	?	9	-19	7/2/13	1.00 p.m.
17 b			11						8	-24		9.00 p.m.
18 a	Shop	Earth floor, 2 windows on either side, kang on both sides	3	10	21	7	Stove with chim-ney	?	11	-16	do.	2.00 p.m.
18 b			2						15	-24		8.00 p.m.
19 a	Native Inn	Plank floor, kang on both sides, 4 paper windows	11	28	19	9	Stove with chim-ney	?	13	-17	do.	2.00 p.m.
19 b			11						14	-23		8.00 p.m.
20 a	Native Inn	Earth floor, kang on both sides, 8 windows	11	28	19	9	Open charcoal pan	?	11	-18	do.	3.00 p.m.
20 b			11						14	-24		9.00 p.m.
SERIES E.												
21 a	Native Inn	Earth floor, kang on both sides, 2 paper windows	12	19	16	7	Charcoal pan	?	14	-18	8/2/13	2.00 p.m.
21 b			13						16	-22		7.00 p.m.
22 a	Native Inn	Plank floor, paper windows	9	40	19	9	Stove with chim-ney	?	12	-18	do.	3.00 p.m.
22 b			12						19	-23		8.00 p.m.

Table of temperature observations in Fuchien (Harbin) (continued).

SERIES E. (cont.).

No. of obs.	Variety of dwelling	Description of building	No. of persons at the time	Approx. size of room, in feet L. B. H.	How heated	Did plague occur here? few or many?	Temp. inside room, C.	Temp. outside room, C.	Date	Time of day taken
23 a	Native Inn	Earth floor, kang on both sides of room, lighted once every evening	12	19 19 9	Charcoal pan, no chimney	?	-14	-14	9/2/13	1.00 p.m.
23 b			11				2	-23	10/2/13	2.00 a.m.
24 a	Native Inn	Earth floor, kang on both sides, burned once every evening, paper windows	7	16 19 9	Charcoal stove with chimney	?	8	-15	9/2/13	12.30 p.m.
24 b			7				5	-23	10/2/13	2.00 a.m.
25 a	Native Inn	Earth floor, kang on both sides, paper window on one side	16	21 19 9	Coal stove with chimney	?	11	-15	9/2/13	3.00 p.m.
25 b			18				13	-23	10/2/13	12.30 a.m.
26 a	Native Inn	Earth floor, kang on both sides	12		Coal stove	?	8	-15	9/2/13	3.00 p.m.
26 b				19 19 9			14	-23		12.00 p.m.
SERIES F.										
27 a	Native Theatre	Wooden floor, all windows on top, natural vent	About 900	85 90 65	2 big brick stoves each $6 \times 3 \times 3$	Yes, many	11	-22	9/2/13	11.00 p.m.
28 a	Native Inn Room A	Earth floor, kang on both sides, lighted once every evening, burn straw, 2 paper windows, dome-shaped roof	15	19 19 9	One charcoal pan without light	?	10	-10	10/2/13	11.00 a.m.
28 b			13		One burning		5	-20	11/2/13	2.00 a.m.
29 a	Native Inn	Do.	7				5	-10	10/2/13	11.30 a.m.
29 b	Room B		16	19 19 9	Only one charcoal pan, unlighted	?	12	-20	11/2/13	2.00 a.m.
30 a	Native Inn	Do.	20		No stove or pan, but kang on both sides	?	12	-10	10/2/13	12.30 p.m.
30 b	Room A		16	19 19 9			11	-20	11/2/13	1.30 a.m.
31 a	Native Inn	Do.	10		One charcoal pot with no chimney	?	11	-10	10/2/13	12.30 p.m.
31 b	Room B		11	19 19 9	Burning		10	-20	11/2/13	1.30 a.m.

32	Native Theatre	As obs. 27	...	650	85	90	65	Obs. 27	Yes	9 d'stair - 6 11 upstairs	16/2/13	2.00 p.m.
SERIES G.												
33 a	Private Room A	Earth floor, kangas on both sides, 2 paper windows		3	9	14	7	Charcoal pan, lighted	Yes	10	- 7	11/2/13 4.00 p.m.
34 a	Private Room B	Same as above, dome-shaped roof		11	19	19	9	Charcoal pan, but without fire	Yes	11	- 7	do. 4.00 p.m.
35 a	Carpenter's shop	Earth floor, kangas both sides, one window papered		5	19	12	...	No stove or charcoal pan whatever	Yes	0	- 9	do. 5.00 p.m.
36 a	Cake shop	Same as above	...	2	19	9	7	Only kang	Yes	6	- 6	do. 2.00 p.m.
37 a	Private	Same as above	...	4	19	9	...	Only kang	Yes	4	- 6	do. 2.30 p.m.
38 a	Lodging House	Earth floor, 6 paper windows	...	8	33	19	...	Kangas, charcoal pan with fire	Reported to have occurred	10	- 7	12/2/13 1.00 p.m.
38 b				18						6	- 19	13/2/13 1.30 a.m.
SERIES H.												
39 a	Eating House	Earth floor, 2 paper windows, kangas on either side		5	16	16	7	Charcoal pan, burning	Yes	1	- 7	12/2/13 2.00 p.m.
39 b				5						5	- 19	13/2/13 1.30 a.m.
40	Private	Same as above	...	13	21	21	7	Stove with chimney, not lighted	Yes	12	- 7	12/2/13 2.00 p.m.
41	Private	Same as above	...	4	23	9	7	Charcoal pan, burning	Yes	8	6	do. 3.00 p.m.
42	Private	Earth floor, kang on both sides, 2 paper windows		3	9	16	9	Charcoal pan, burning	Yes, many	13	- 7	13/2/13 4.00 p.m.
43	Private	Earth floor, kang on both sides, 1 window		2	16	9	12	Stove with chimney, no fire	Yes, many	14	- 6	do. 5.00 p.m.
44	Small Inn	Same as above, but brick wall	...	2	9	19	7	Stove with chimney, burning	Yes, over 10 persons	16	- 6	do. 3.00 p.m.
45	Private	Plank floor, kang on one side, glass windows		6	Chimney stove with fire		18	- 7	do. 4.00 p.m.

Table of temperature observations in Changchun.

SERIES A.										
No. of obs.	Variety of dwelling	Description of building	No. of persons at the time	Approx. size of room, in feet. L. B. H.	How heated	Did plague occur here? Few or many?	Temp. inside room, C.	Temp. outside room, C.	Date	Time of day taken
1	Chinese Hotel	Plank floor; one glass window at the back wall, an entire glass window in front wall open to the yard. A kang inside room	1 Med. Off. himself	22 11 12	1 kang, charcoal pan burning, a stove with chimney extending from next room	No. Built after the epidemic	7	- 3	21/2/13	1.00 a.m.
2	Chinese Theatre	Plank floor; a "U" shaped amphitheatre facing the stage, glass windows on all sides but always closed in cold weather. About 600 seats	About 370	150 70 50	3 stoves with chimney at corners of ground floor	No. Built after the epidemic	5	- 3	21/2/13	6.00 a.m.
3	Small Hawker's dwelling	Earth floor; oppos. kangs; dark; 2 paper windows about 2 sq. ft. each	7	13 19 8	1 small charcoal pan kept burning	Yes	- 3	- 7	22/2/13	3.00 p.m.
4	Hawker's shop	Earth floor; 1 kang, 2 sky-lights, 2 paper windows. Part of room used for cooking	7	19 13 6	Earthen pot burning charcoal	Yes	1	- 7	22/2/13	3.00 p.m.
5	Coolie hut	Earth floor; one side of the room used. Kaoliang stalks as a partition. 2 paper windows; 1 kang	13, all on the kang for warmth	19 13 8	No stoves, kang lighted once or twice a day for food	Yes	1	- 8	22/2/13	3.00 p.m.
The houses of obs. 3, 4, and 5 are situated 2 miles from Changchun, so it was not easy to make the second observation.										
SERIES B.										
6	Public Bath	Cement floor; glass windows in front wall, wooden benches along all sides of wall; bright but air-tight	12	34 20 11	Chimney stove burning coal	Yes, taken at corner over 30 of room	23	- 8	22/2/13	4.00 p.m.
7	Chinese Inn	Earth floor; 1 kang, wooden partition dividing room into two portions, 2 paper windows behind the kang	10	9 8	Stove burning coal in one of the rooms	Yes, but not this room; the infected room is now locked up	13	- 8	22/2/13	4.30 p.m.
8	Chinese Inn	Plank floor, paper window in front, 2 kangs opposite each other, one was heated	10	23 12 10	Stove with chimney burning coal	No	21	- 8	22/2/13	5.15 p.m.
			7				17	- 15	23/2/13	12.30 p.m.

9	Chinese low class Inn	Earth floor, damp, dark; 3 kang; paper window; dome-shaped roof	11	18	22	10	Stove with chimney, burning	No	16	- 8	23/2/13	12.30 p.m.	
10	Private Residence	Earth floor, dark and damp; 2 kang, kaoliang (millet) stalk roof; conical shape; paper window, half room used as kitchen	20	5	17	21	9	No stove but a square brick stove for cooking	Yes, all in house were carried off by plague	16	- 19	24/2/13	2.00 a.m.
			6						0	- 4	23/2/13	1.00 p.m.	
									1	19	24/2/13	2.00 a.m.	
SERIES C.													
11	Chinese Inn	Plank floor, paper window, walls lined with paper have not been torn down after the epidemic, but have had a little alteration since then	6	20	13	9	Stove with chimney extending into it from next room	Yes	9	- 8	23/2/13	1.00 p.m.	
12	Police Station	Earth floor; oppos. kang; paper window, damp and dark; mud wall, kaoliang stalk roof	18	37	18	10	No stove whatever, kang burned twice a day	Yes, 24 out of 25 persons	11	- 5	24/2/13	2.00 p.m.	
13	Chinese low class Inn	Earth floor, oppos. kang; street wall has 2 paper windows about 3 sq. ft., 1 window at back wall, paper ceiling, mud wall	29	11	16	16	9	Only 2 kang that receive warmth from 2 cooking stoves outside	Yes	3	14	25/2/13	6.00 a.m.
14	Chinese low class Inn	Earth floor, 3 kang like a □ shape; oppos. walls, both were paper lined, 3 square brick stoves attached to each of the kang, old and damp	16	13	32	33	11	Only the 3 brick stoves for cooking	Yes, about 3 or 4 persons lost their lives here	5	- 5	24/2/13	3.00 p.m.
15	Chinese low class Inn	Earth floor, oppos. kang; paper window on street side, old and wet	24	5	20	17	9	An open charcoal pan	Yes	3	- 14	25/2/13	6.00 a.m.
									5	- 5	24/2/13	3.30 p.m.	
SERIES D.													
16	Chinese Inn	Earth floor, shaped kang, 1 paper window, 1 brick stove for cooking. Kaoliang stalk roof	20	20	34	20	10	One charcoal pan, burning	Yes	8	- 6	25/2/13	5.00 p.m.
17	Wagon maker	Earth floor, 3 paper windows on one side, damp, 3 flat cooking stoves, mud wall	29	25	24	22	10	Only the 3 stoves attached to the kang, burned when cooking	Yes	6	- 18	26/2/13	2.00 a.m.
									9	- 6	25/2/13	5.00 p.m.	
			23						9	18	26/2/13	3.00 a.m.	

APPENDIX IV.

*Temperature Observations in Fuchiatien (Harbin) and
in Changchun.*

In reply to a request made by Dr Oscar Teague (American delegate to the Mukden Plague Conference) for particulars of temperatures inside and outside houses of towns where the epidemic of 1910-11 had been most severe, *e.g.* Fuchiatien, Changchun, the observations comprising this Appendix were made under the personal supervision of Dr T. N. Tang, Assistant Medical Officer. The following is a summary of the recorded on pp. 284-289 data:

1. *Re Observations in Fuchiatien.*

1. The native dwellings are heated by :
 - (a) brick stoves after the Russian style (very few);
 - (b) iron stoves in which coal is usually burnt;
 - (c) *K'angs*, *i.e.* large rectangular mud and brick structures 2 feet above the ground on which the people sit and rest, heated usually by millet stalk;
 - (d) open charcoal pans without chimneys.
2. The windows consist usually of a wooden framework pasted with thin white paper, thus letting in very little light.
3. Besides the doors and the cracks in the windows, walls, and roofs, there is seldom any ventilation inside the dwellings.
4. The following are some of the more interesting observations :
 - (a) Period of observation, February 2nd to 13th, 1913.
 - (b) Number of dwellings examined 44.
 - (c) Number of observations taken 75.
 - (d) Highest temperature recorded

{	outside	- 2° C.
{	inside	22° C.
 - Lowest " " "

{	outside	- 24° C.
{	inside	0° C.
 - (e) Average " "

{	outside	- 11.5° C.
{	inside	11.7° C.

2. *Re Observations in Changchun.*

- (a) Period of observation, February 21st to 26th, 1913.
- (b) Number of dwellings examined 17.
- (c) Number of observations taken 28.
- (d) Highest temperature recorded

{	outside	- 3° C.
{	inside	23° C.
- Lowest " " "

{	outside	- 20° C.
{	inside	- 3° C.
- (e) Average " "

{	outside	- 10.2° C.
{	inside	8° C.

THE INACTIVATION OF COMPLEMENT BY MECHANICAL AGITATION.

By HANS SCHMIDT.

(*From the Bacteriological Department, Lister Institute, London.*)

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INTRODUCTORY.

OF the many methods employed to render a serum inactive, that by means of mechanical agitation has been the subject of several recent works, but the real nature of this phenomenon, as it occurs in shaken sera, is still unknown. The reason is the uncertainty regarding the nature of complement itself, and even the most satisfying explanation of the inactivation of complement by shaking would not probably throw much light on the nature of the complement. I give in the following

a brief summary of the literature dealing with the inactivation of complement by mechanical agitation.

The first systematic experiments to render a serum inactive by shaking were undertaken by Jakoby and Schuetze (1909). These authors found (1910) that mechanical agitation renders complement inactive, the time necessary for the effect being shorter the higher the temperature at which the shaking takes place. By this procedure the serum becomes dim and a precipitate occurs, but sera which were not completely inactivated, also showed dimness, so that the authors concluded that the inactivity cannot be due solely to the coagulation and precipitation of proteins. A serum does not lose the property of being inactivated by shaking if it is frozen and freezing does not reactivate a shaken inactive serum. The latter can however be reactivated by both fractions of the complement either by end-piece or by mid-piece, and if the serum is centrifugalised the precipitate can be reactivated by both fractions, while end-piece only is capable of reactivating the supernatant fluid. Shaken inactive serum does not inhibit normal complement action. The experiments of these authors were in part confirmed by Zeisler (1909), Stuehmer (1910), Noguchi and Bronfenbrenner (1911). These papers however did not add new facts to those already known by Jakoby and Schuetze, but an elaborate paper of Ritz (1912) gave to many points a better explanation. Ritz, with a better shaking apparatus, succeeded in shortening considerably the time necessary for inactivation, so that 20 to 30 minutes shaking was sufficient, while Jakoby and Schuetze were obliged to shake six hours and longer to render a serum inactive. He confirmed also the accelerating action of the temperature. Jakoby and Schuetze took into consideration the difference between ordinary and Jena glass as affording some explanation of the fact, that some sera could be inactivated in a relatively short time while others were very resistant to the shaking effect, and they believed that the discrepancy might be due to the alkali produced from the ordinary glass. Ritz (1912) however accentuated the important rôle, which the relation between the volume of the tube and the quantity of the shaken liquid plays in the process. He proved experimentally that even small differences (100–110 c.c.) between the volumes of different tubes—the quantity of shaken liquid being the same—influence very greatly the time necessary for inactivation by shaking, thus believing that the variant results in the experiments of Jakoby and Schuetze are due to these circumstances. Ritz found by examining the relation between the volume of the tube and the quantity and concentration of the liquid,

that with a concentration 1:10 of serum the rapidity, with which inactivation by shaking is achieved, is optimal. With regard to the reactivation of shaken inactive serum by the complement fractions, Ritz was able to confirm the results of Jakoby and Schuetze, but he showed that this reactivation depends upon the time during which the serum is exposed to agitation. Long periods of shaking render the serum incapable of being reactivated. He further showed, contrary to Jakoby and Schuetze, that the precipitate cannot by any means be reactivated. In some experiments Ritz succeeded in reactivating shaken inactive serum by adding thermoinactive serum, but this effect of the latter is reduced to a minimum if the shaking is continued for a long time. This phenomenon has been confirmed by Kashiwabara (1913), but not in all sera. The time he used for thermoinactivation was always half an hour at a temperature of between 50.7° and 60° . This exposure to heat is probably too long and deprives the serum of its property to reactivate shaken serum. In further experiments Kashiwabara showed, that the reactivation of shaken serum by mid- or end-piece is no longer possible if these fractions have been separately shaken before use. In a mixture of one fraction shaken with the other unshaken, complement action however can be restored, if the latter fraction is sufficiently concentrated. The author found, that thermoinactive serum, if shaken before or after the treatment by heat, loses its property of reactivating shaken-inactive serum. Finally I would mention the experiments of Courmont and Dufour (1912). These authors (1912) showed, that sera inactivated by shaking do not become anticomplementary like sera inactivated by heat. Experiments undertaken with the object of studying the influence of the gas with which the serum is shaken led the authors to the conclusion, that the oxygen plays an important part in the inactivation.








It is chiefly with the object of ascertaining whether confirmation could be obtained of this important statement of Courmont and Dufour that the following investigations were undertaken.

Technique of experiments.

Not having at my disposal an Uhlenhuth's kinotherm such as Ritz used for his experiments, I employed an ordinary shaking apparatus, the electro-motor of which was provided with a resistance to regulate the velocity. Further the apparatus was fitted with an arrangement for altering the distance, along which the shaken tube was moved. In the following experiments this distance was always 7.8 cm. The serum was

Inactivation of Complement

shaken in a dark room at a temperature of between 33° – 36° C. I used cylinder-shaped tubes closed by rubber stoppers. Many controls showed me, that the contact of the serum with the carefully cleaned rubber did not alter the properties of the serum. Only the haemolytic complement of guinea-pig serum has been examined. Its complementary function was tested by its haemolytic power in combination with sensitized, three times washed, sheep red corpuscles of which a 5% emulsion in 0.85% saline solution was used. The amboceptor was inactivated rabbit serum, the single lysing dose of which was 0.00125 c.c. by using 1 c.c. of the red cell emulsion and 0.1 c.c. complement. In the haemolytic tests the following scheme is used to illustrate different degrees of haemolysis.

No haemolysis	
Trace haemolysis	
Slight	„	...	
Half haemolysed	
Strong haemolysis	
Almost complete haemolysis	
Complete haemolysis	

To split the complement-containing serum into the so-called mid- and end-piece I used the procedure of Sachs and Altmann (1908) in the following way:

1.0 c.c. undiluted complement serum plus 8.2 c.c. $n/300$ HCl in aqu. dist. was kept for 1 hour at room temperature, then centrifugalised and the sediment instantly—to avoid Brand's modification—washed three times with distilled water. 10 c.c. of 0.85% saline solution were then added. The supernatant fluid, after being filtered, was neutralised and rendered isotonic by adding 0.8 c.c. of an $n/30$ (NaOH) solution which contains 10% saline. The result is that both fractions are in a dilution of 1:10 and that the mixture of 1.0 c.c. of each corresponds to 0.1 c.c. of each original complement-containing serum. I obtained good results by this procedure in most cases. Sometimes the supernatant fluid (end-piece) still retained some lytic property, but it could be avoided by allowing the serum to remain 15 minutes to half an hour longer in contact with the hydrochloric acid.

Influence of time, temperature and proportion between volume of tube and volume of fluid.

The dependence of the shaking inactivation upon time and temperature could be confirmed as follows: at low temperatures, 0–20°, shaking must be continued a very long time before the complement action is perceptibly weakened. Occasionally fresh serum is practically not affected by shaking at low temperature. The time necessary for inactivation by shaking decreases rapidly with rise of temperature. In my experiments however the average time required to render the serum completely inactive was 4–6 hours at 33° C. Other factors however, such as the concentration of the serum and the relation between the volume of the tube and the quantity of shaken liquid, influence greatly the time necessary for inactivation. Ritz (1912) had already laid much stress on these factors. I too attempted to determine experimentally the optimum of the concentration and of the relation between the volumes of tube and liquid in regard to the shaking inactivation, but I met with considerable difficulties, as I shall explain in the following:

The tubes I used for shaking were cylinders of different width and length. Now if the temperature, the velocity, and the distance of the shaking movement, as well as the diameter and the length of the tube are kept constant, there are still two variables left, viz. the concentration of the serum and its quantity, and, as a function of both, the absolute amount of complement. The latter is naturally altered, if the quantity of the serum is kept constant and the concentration varies, but if the concentration of the serum is kept constant and the quantity changed, the absolute amount of complement is still different in each tube. If instead of a serum, containing complement in a certain concentration, only pure water is considered, the problem is reduced, so that it can be more easily treated from a purely physical standpoint. Thus the problem would be the following:

• In a given cylindrical vessel, water is to be shaken with air under constant conditions. What must be the relation between the quantity of water and the volume of the tube to render the intensity of shaking a maximum?

This problem is not soluble experimentally because no criterion exists to judge the degree of shaking intensity. But also theoretically I was unable to find a solution of the problem because there are still other relations to be taken into account, as that between the length of

the tube and that of the liquid column in it, as well as the range of the shaking movement, and further the relation between the diameter of the tube and the viscosity of the liquid and the velocity of the motor. If the problem is already very complicated in the case of water, it is still more so in that of serum, for a froth will be formed which keeps the air included in its bubbles and so prevents the air in a certain degree from getting through the serum. Especially disturbing is the different absolute amount of complement, which results in varying either the quantity or the concentration of the serum. However, in the case of serum, which is being shaken, the succeeding inactivation offers a certain measure of the intensity of shaking. In order to render it possible to have the absolute amount of complement constant in each tube and at the same time to vary the concentration, I took cylinders of the same diameter and different length but such that the relation of the length to the volume of the liquid was the same in all tubes. But now the relation of the tube-length to the shaking range is varied and renders an exact comparison impossible. It would appear evident that this problem is not capable of exact treatment by experiment unless several shaking apparatus are used at the same time, which allow the shaking distance to be altered, while all other conditions can be kept constant. However, my experiments allow some qualitative conclusions to be drawn and therefore some of them may be reproduced as follows:

Experiments regarding the influence of concentration and of the proportion between volume of tube and volume of liquid on the inactivation by shaking.

EXPERIMENTS I—VI.

























EXP. I. Guinea-pig serum $\frac{1}{10}$ diluted is shaken in tubes of 20 c.c. volume $5\frac{1}{2}$ hours at 36° . 160 vibrations in a minute.

[Equal concentration—different quantities of serum.]



























Control	I	II	III	IV	V	VI
Quantity of $\frac{1}{10}$ serum...			15 c.c.	10 c.c.	8 c.c.	5 c.c.	3 c.c.	2 c.c.
Haemolysis: (1 c.c. $\frac{1}{10}$ compl. + 1 c.c. of sens. red corpuscles. AB dose = 0.0025.)			■	■	■	□	□	□

Tube I showed no precipitate, tube II only a slight amount, while in all other tubes a thick precipitation could be observed.


















































EXP. II. In cylinders of 50 c.c. volume different quantities of $\frac{1}{10}$ diluted guinea-pig serum is shaken 3 hrs. at 36° .

		Control	I	II	III
Quantity of compl. $\frac{1}{10}$...	35 c.c.	10 c.c.	5 c.c.
Looking after 3 hrs. shaking...		Clear	Very slight dimness	Dim	Thick precipitate
Haemolysis : (1 c.c. sens. red corp. AB dose = 0.0025.)	$\frac{1}{10}$ compl. c.c. 1.0				
	0.75				
	0.5				
	0.25				
	0.1				
	0				

EXP. III. In cylinder-shaped tubes of 35 c.c. vol. equal quantities (12.7 c.c.) are shaken 5 hours at 35° , the concentration being varied.

		Control	I	II	III	IV	V	VI
Concentration of compl. ...		$\frac{1}{10}$	$\frac{1}{2}$	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$
$\frac{1}{2}$ complement ser. c.c.	1.27	6.35	3.175	2.1	1.27	0.84	0.63
0.85 % NaCl c.c.	11.43	6.35	9.525	10.6	11.43	11.86	12.1
Haemolysis after 5 hrs. shaking. (1 c.c. sens. red cells AB dose 0.0025 c.c.)	Compl. 0.1							
	0.05							
	0.025							
	0							

EXP. IV. Guinea-pig serum was shaken $2\frac{1}{2}$ hours at 36° in tubes of the same length = 15.2 cm., the same diameter = 13 mm. (volume $v=20.17$ c.c.). The serum was diluted to $\frac{1}{10}$ and the height of the serum standing in the tubes is indicated by h , while the serum quantity is indicated by m .

		Control	I	II	III	IV	V	VI
h		10.13	7.6	5.06	3.8	3.04	1.52	
$\frac{v}{m}$		1.5	2	3	4	5	10	
Haemolysis : (1 c.c. sens. red cells. AB dose = 0.0025 c.c.)	$\frac{1}{10}$ compl. c.c. 1.0							
	0.75							
	0.5							
	0.25							
	0.15							
	0.1							
	0							




































































Inactivation of Complement

Exp. V. Seven tubes with the following measures

	Diameter	Length	Volume
I	2.7 cm.	17.0	95
II	1.88	17.1	47
III	1.58	16.8	32
IV	1.3	15.2	20
V	1.0	16.7	15
VI	0.85	16.5	9.5
VII	0.68	15.6	5.5

were filled with $\frac{1}{10}$ diluted guinea-pig serum to $\frac{1}{4}$ of their length. After two hours shaking in all tubes precipitation occurred, but from tubes I to VII there were an increasing precipitation and a decreasing dimness, so that in tube I the serum was very dim but only a slight precipitate on the bottom, while in tube VII the serum looked quite clear with thick floating coagula.

Haemolysis with 1 c.c. sens. red corpuscles. AB dose=0.0025 c.c.












$\frac{1}{10}$ compl. serum, c.c.	1.0	0.75	0.5	0.25	0.15	0.1	0.05	0
Untreated serum ...								
Control serum ...								
I								
II								
III								
IV								
V								
VI								
VII								

Tube IV had a different length compared with the other tubes, which may be the reason for the different effect of shaking.

Exp. VI. Fresh complement in different concentration was shaken in different tubes such that the absolute amount of complement was the same in each tube. [34°, 6 hours shaking.]

	I	II	III	IV	V
Tube volume (v) ...	4	10	16	20	30
Serum quantity (m) ...	1.6	4.0	6.4	8	12
Concentration ...	$\frac{1}{2}$	$\frac{1}{5}$	$\frac{1}{8}$	$\frac{1}{10}$	$\frac{1}{15}$
Absolute amount of original compl.ser.	0.8	0.8	0.8	0.8	0.8
$\frac{v}{m}$	2.5	2.5	2.5	2.5	2.5

After 6 hours shaking, all serum is brought to $\frac{1}{15}$ dilution with saline. Haemolysis with 1 c.c. sens. red corp. AB dose = 0.0012 c.c.

$\frac{1}{15}$ compl., c.c.	1.0	0.5	0.25	0.15	0.1	0
Control	...					
I						
II						
III						
IV						
V						

Analysis of experiments I-VI.

From these experiments it may be concluded, that a dilution of 1:10 gives better results in regard to shaking inactivation than any higher concentration, but in some experiments equally good results were obtained by using dilutions of 1:15 and 1:20, so that I refrain from speaking of an optimum. With regard to the already mentioned relation between the volume of the tube and the quantity of the shaken serum the best results are obtained if the quantity of the latter is as small as possible compared with the total volume of the tube, but there must be enough serum present to prevent it from becoming entirely converted to froth, which is then no longer exposed to the same intensity of agitation. In another paper (Schmidt, 1913), some remarks were made as to the necessity of taking the froth into consideration in measuring the surface-tension, but so far as complement action is concerned, I did not succeed in tracing any difference in the lytic action of the froth or of the remaining liquid. Generally speaking, the smaller the quantity of shaken serum is, in comparison with the tube volume, the sooner dimness and the formation of coagula occur, which in my opinion represent the first stage of the inactivation. I did not succeed in determining a definite relation between the volumes, for there are so many unforeseen circumstances that in order to explain such differences individual differences in the guinea-pig sera must be presumed.

The precipitate in shaken serum.

Jakoby and Schuetze (1910) observed the formation of precipitates in the shaken serum, which they found to have no direct connection with the complement inactivity. Ritz (1912) wrote in a footnote to his

paper, that the precipitation runs to some extent parallel with the inactivation but did not exactly correspond with the latter.

On summarising my experiments I can say that I have never seen a serum inactivated by shaking without a precipitate occurring previously, but many sera were observed to be dim and filled with floating coagula. These sera were however not yet inactivated, although corresponding to the intensity of the coagulation they were distinctly weakened in regard to their complement function. I therefore conclude, that the precipitation must in time precede the inactivation, which latter can never take place without the formation of the first. Now the question arises, what that precipitate consists of.

W. Ramsden (1894) showed that shaking of different protein solutions effected a partial separation of the soluble substance in the form of membranes, and that it was possible by means of enlarging the surface of the protein solutions with respect to the gas, as in the process of shaking, to cause almost the total amount of protein of a diluted egg-white solution to coagulate and separate. According to Ramsden (1903, 1904), protein lowers the surface-tension of water and is therefore adsorbed on surfaces and thus relatively concentrated. This increase of concentration in the surface leads to aggregation of particles of protein, before isolated, and further to a diminution of the specific surface of the particles, which is followed by sedimentation due to gravitation and depending upon the viscosity of the solution. The presence of a free surface against gas is necessary to produce this form of coagulation, according to Ramsden (1903, 1904). But the nature of the gas has no influence in so far as it is chemically inactive. With respect to the sera, which are solutions of protein, it is evident, that by shaking, similar phenomena must be observed, especially as the surface tension of serum is lower than that of water and sera possess the tendency to form a relatively high surface viscosity, which enables them to produce stable froth. Now shaking produces also in sera aggregation of proteins and coagulation, which leads to a partial separation of the protein in the form of a precipitate. After removing the precipitate and shaking again a new precipitate is formed. In another paper (Schmidt, 1913) I was able to show that during this procedure the surface tension is not lessened as in the case of thermoinactivation and coagulation by heat.

It appears evident therefore that the precipitate produced by mechanical agitation represents nothing else than mechanically coagulated protein. Ramsden also observed the formation of coagula in shaken

sera, but he noticed that most of them went into solution again, only a part of the coagula remaining insoluble. It takes therefore much longer time to produce a mechanical coagulation in sera than in solutions of egg-white. According to Ramsden the coagula thus produced in sera consist of unaltered serum albumin. With regard to the relation of this precipitate to complement, Jakoby and Schnetze state that they observed a reactivation of such precipitate, although in a slight degree, by each of the two complement fractions. Ritz (1912) could not confirm this statement and I too must agree with Ritz. I found the precipitate to be insoluble either in 0.85% saline solution or in distilled water or in serum, even if heated to 56°. But the precipitate disappears from the eye by adding some KOH, a phenomenon which, according to Ramsden, does not imply solution of the precipitate, which is merely soaked. I found neither end-piece nor mid-piece to be activated by the precipitate, even in the slightest degree. I am inclined to identify this precipitate with that, which is often seen in sera, which is kept in a cold room for a long time and which I have found to be also insoluble even by heating to 56°.

Influence of the nature of the gas on serum.

Provided that the gas in which the serum is shaken is chemically inactive its nature does not play any part, according to Ramsden. The latter shook protein solutions also *in vacuo* and obtained the same phenomenon of coagulation. But recently Courmont and Dufour (1912 *b*) published experiments, in which they claim to have obtained the shaking inactivation of complement-containing sera much quicker, if the serum has been shaken in oxygen, whereas no alteration of the complement function took place, if the serum was shaken in nitrogen or *in vacuo*. The authors believe it to be very probable that the inactivation by shaking in air is of the nature of an oxidation process. In order to examine, how far complement action is influenced by the presence of oxygen I undertook a series of different experiments. First of all I studied the influence of oxygen on thermoinactivation.

(a) The influence of oxygen on inactivation by heat.

















































































































For this purpose serum, undiluted as well as diluted to 1:10 by saline solution, was evacuated and then the vacuum filled with hydrogen. After shaking the serum several times in hydrogen it was again evacuated and then again hydrogen allowed to enter. This procedure was

Inactivation of Complement

repeated seven times and finally the serum was sealed in an atmosphere of pure hydrogen. It can confirm Simnitzki's (1903) statement, that the removal of oxygen does not influence the complement action, the haemolysing effect being the same as before. As control to this procedure the same serum was saturated with pure oxygen by shaking it repeatedly in the gas and then sealing it in a glass tube containing pure oxygen. The sera treated in this way were exposed to the temperature of 56° , the time being varied. The result of the thermo-inactivation thus produced can be seen from the following table:

The influence of oxygen on thermoinactivation.

Exp. VII. Each tube, containing 1 c.c. complement serum, was exposed to heat of 56° during different times under H and O. Haemolysis with 1 c.c. sens. red corp. AB dose = 0.0025 c.c., the total volume in each tube being 2 c.c.

Compl. serum $\frac{1}{2}$ c.c.	1.0	0.75	0.5	0.25	0.15	0.1	0.05	0
Time of exposure to 56° , mins. } ... 0								
1								
2								
3								
4								
5								
6								
0								
1								
2								
3								
4								
5								
6								

The table shows that not the slightest difference in the effect of heat on complement-containing serum resulted whether the serum was exposed to heat under much oxygen or without oxygen. In an astonishingly short time (cf. Husler (1912), H. Schmidt (1913)) the serum was inactivated in both cases.

(b) *The influence of oxygen on the storage of complement.*

I made further investigations as to the influence of oxygen in inactivating complement which had been stored. For this purpose I treated sera as above mentioned and kept them under oxygen, nitrogen and hydrogen for days and weeks, in most cases in the cold room but sometimes at room temperature and at 37°. In some experiments I allowed oxygen and hydrogen to blow continuously over the serum for 24 hours at 37°. I can give a brief summary of all these experiments by saying that I never succeeded in demonstrating unequivocally, that complement can be preserved longer in the absence of oxygen than with it. In experiments, which seem to give this result, larger bacterial growth could be observed in the serum kept under oxygen. Such sera produced a certain violet colour in all test-tubes whether haemolysis occurred or not. This phenomenon is due to bacterial growth, according to Kostrzewski (1911). K. Hara (1913) showed that complement can be preserved longer if kept sterile and that bacterial growth causes the disappearance of the complement and the anticomplementary property, which can be observed in some sera. I succeeded however in keeping the complement-containing serum in a slight degree of activity for about four weeks but I could not find the complement to be preserved longer in the absence of oxygen. In fact no difference could be traced.

(c) *The influence of oxygen on inactivation by shaking.*

Special investigations were then made in regard to the inactivation by means of shaking. Courmont and Dufour shook complement-containing serum *in vacuo*, giving 6 mm. Hg pressure. I refrained from using a vacuum, because in an even stronger vacuum of air as these authors used, enough molecules of oxygen are still present to effect eventually an oxidation, and I appeal to the fact that Ramsden saw coagulation occurring in protein solutions, which were shaken *in vacuo*. I shook the sera in different gases as oxygen, hydrogen and nitrogen at the same time varying the conditions with regard to concentration and relation between the volumes of the tube and of the serum. In the case of hydrogen and nitrogen employed care was taken to ensure complete removal of all oxygen. I can also here summarise my results by saying that I observed in every case the formation of coagula and that the greater the attention paid to render the external conditions as constant as possible, the more the results—in the beginning often found to be contradictory—became the same. In the earlier experiments I often

obtained confirmation of the experimental results of Courmont and Dufour, that is to say, destruction of complement, if it is shaken in oxygen and only a slight alteration of the activity when shaken in hydrogen or nitrogen. But sometimes I found the contrary, complete destruction in nitrogen or hydrogen and almost none in oxygen. But the more the conditions, under which the sera were shaken, were rendered equal, the less was the difference in the degree of inactivity obtained, and finally no more difference could be observed in the haemolysis produced by complement-containing serum whether shaken in oxygen or in hydrogen or nitrogen.

I give in the following a few of my experiments:






















Experiments showing the influence of the nature of the gas on inactivation by shaking.

EXPERIMENTS VIII—XI.

















































Exp. VIII. Fresh complement serum in $\frac{1}{10}$ dilution was shaken for 6 hours at 36°. The relation between the tube volume (35 c.c.) and the serum quantity was kept constant=2. Haemolysis with 1 c.c. sens. red corp. AB dose=0.0025 c.c.

$\frac{1}{10}$ compl. serum, c.c.	2.0	1.0	0.75	0.5	0.25	0.15	0.1	0
Control								
Oxygen								
Nitrogen								
Air								























































Exp. IX. 3 c.c. $\frac{1}{10}$ serum is shaken 4 hours at 37° in tube of 12 c.c. volume.

Compl. ($\frac{1}{10}$), c.c.	1.0	0.5	0.25	0.15	0.1	0.05	0
Control							
Oxygen							
Nitrogen							

Exp. X. $\frac{1}{10}$ compl. serum 4 hours shaking at 36°.

Tube volume (v)	Quantity of $\frac{1}{10}$ compl.	Gas	c.c. of $\frac{1}{10}$ complement serum							
			2.0	1.0	0.75	0.5	0.25	0.15	0.1	0
24	8	air								
47	15	O								
50	15	H								
Control serum		air								
"	"	O								
"	"	H								

EXP. XI. $\frac{1}{10}$ complement serum is shaken under conditions as equal as possible. Tube volume 20 c.c. Complement serum quantity 8.5 c.c.

$\frac{1}{10}$ compl. ser., c.c.	2.0	1.5	1.0	0.75	0.5	0.25	0.15	0.1	0	Time of shaking
O										1 hour
H										1 hour
O										2 hours
H										2 hours
O										3 hours
H										3 hours

Courmont and Dufour (1912 *b*) also showed a slight loss of complement action occurring in the sera shaken *in vacuo* or with nitrogen in comparison with the control. I am inclined to consider the results obtained by these authors in regard to shaking inactivation as experimental errors caused by slight alterations in the external conditions but sufficient to render the intensity of the shaking agitation different in each case. That small alterations in the relation between the volumes of the tube and the liquid are still capable of giving variant results is shown by Ritz (1912), and I too had plenty of opportunity of being convinced of these facts (cf. Exp. X).

Now this result agrees with that obtained by Ramsden (1904), who said that the nature of the gas is of no importance to the mechanical coagulation in protein solutions. However, as a sort of *experimentum crucis* I mention the following experiment:

After removing the oxygen as completely as possible from a serum by a procedure similar to that above mentioned, I added carefully cleaned glass beads and closed the tube with a rubber stopper, so that no trace of free gas could be observed even after shaking the tube for many hours. An intense shaking giving 200 vibrations in the minute caused whirlpools in the liquid by the movement of the glass beads. The mechanical agitation thus produced is relatively small compared with the shaking in gases. However, the experiment shows that the complement action was weakened and after shaking a very long time at 36° C. complement was rendered practically inactive, the serum being then very dim owing to the presence of coagulated proteins.

Complement-containing serum shaken with glass beads without any gas.

Exp. XII. $\frac{1}{10}$ serum is shaken for 3 hours at 36°. Tube volume = 47 c.c. The tubes with air, O and H were filled with 12 c.c. $\frac{1}{10}$ complement serum. One tube was partially filled with glass beads and then filled with serum without gas.

c.c. of $\frac{1}{10}$ compl.	1.0	0.75	0.5	0.25	0.15	0.1	0.05	0
Shaken in air ...								
Shaken in oxygen ...								
Shaken in hydrogen ...								
Shaken with glass beads								
Control, in air ...								
Control, in oxygen ...								
Control, in hydrogen ...								

$\frac{1}{10}$ complement serum, 8 hours shaken with glass beads at 36°.

$\frac{1}{10}$ compl. ser. c.c.	1.0	0.5	0.25	0.15	0.1	0
Normal serum ..						
Control serum ..						
Shaken with glass beads						

From these experiments it appears to be evident that inactivity by shaking is due to mechanical influence, and that every factor capable of increasing the intensity of agitation has an accelerating effect upon the destruction of complement by means of agitation. To presume an oxidation by the shaking with air is not necessary and is not in agreement with the observed facts. Shaklee and Meltzer (1909) found also in shaking proteolytic ferments "that the destruction was not due to an oxidation by the oxygen in the air," which was proved by the fact "that the destructive effect remained the same, when the space above the liquid within the bottle was filled with hydrogen."

Reactivation of serum inactivated by shaking by normal complement and its fractions.

It has been shown that the shaking inactivation is associated with a mechanical coagulation and separation of a part of the proteins, and further these coagulated proteins are proved to have no direct connection with the complement. Now the shaken inactivated complement is represented by the supernatant fluid of the centrifuged shaken

serum. I mentioned above that Jakoby and Schuetze (1910) found that fresh end-piece can reactivate this fluid to a large extent, a statement which has been confirmed by Ritz (1912) and Kashiwabara (1913), who further succeeded in reactivating shaken inactive serum by thermoinactive serum. In order to prove these different possibilities of reactivating a shaken inactive serum I undertook some experiments and was able to obtain more or less the same results.

I give a brief record of some of my experiments in the following:

Experiments showing the reactivation of complement inactivated by shaking, by normal complement and its fractions.

Exp. XIII. Normal end-piece and mid-piece are obtained by the procedure of Sachs and Altmann and are each in $\frac{1}{10}$ dilution so that 1.0 c.c. end-piece plus 1.0 c.c. mid-piece correspond with 1.0 of $\frac{1}{10}$ active complement.

	Haemo- lysis
1.0 c.c. n. end-piece + 1.0 c.c. sensit. red corp. + 1.0 c.c. saline—0.85 % ...	□
1.0 c.c. n. mid-piece + 1.0 c.c. „ „ + 1.0 c.c. „ „ ...	□
1.0 c.c. n. mid-piece + 1.0 c.c. „ „ + 1.0 c.c. end-piece ...	■
Shaking precipitate + 1.0 c.c. end-piece + 1.0 c.c. sensit. red corp. ...	□
„ „ + 1.0 c.c. mid-piece + 1.0 c.c. „ „ ...	□
„ „ + 1.0 c.c. n. $\frac{1}{10}$ complement + 1.0 c.c. sensit. red corp. ...	■
Centrifugalised shaken serum + 1.0 c.c. n. end-piece + 1.0 c.c. sensit. red corp. ...	■
„ „ „ + 1.0 c.c. n. mid-piece + 1.0 c.c. „ „ ...	□
„ „ „ + 1.0 c.c. $\frac{1}{10}$ n. complement + 1.0 c.c. sensit. red corp. ...	■
1.0 c.c. $\frac{1}{10}$ complement + 1.0 c.c. end-piece + 1.0 c.c. sensit. red corp. ...	■
1.0 c.c. „ „ + 1.0 c.c. mid-piece + 1.0 c.c. „ „ ...	□

These experiments permit one to make the following statement:

After splitting a fresh complement-containing serum successfully into its two fractions by the method of Sachs and Altmann, I was able to confirm the statement that the supernatant fluid of the centrifugalised shaken inactive serum could be brought to full complement action by adding fresh normal end-piece, but no reactivation occurred by addition of mid-piece. A normal haemolytic system is not inhibited by the presence of shaken inactivated serum, as happens occasionally with thermoinactive serum. I mention the confirmation of this statement, made also by Courmont and Dufour (1912 *a*), because it is remarkable

that the centrifugalised shaken inactive serum had no anticomplementary power in all my experiments, although it acts by its reactivation with end-piece as mid-piece, which latter has sometimes strong anticomplementary action (P. Schmidt (1911), Ledingham and Dean (1912)).


















Reactivation of thermoinactive serum.

To examine further the reactivation of a shaken inactive serum by a thermoinactivated serum I investigated first, how far the latter can be reactivated either by fresh serum or by the complement fractions.

Exp. XIV. $\frac{1}{10}$ complement serum has been inactivated by heating it to 55° during 2, 3, 5, 30 mins. By splitting complement serum the following was obtained:

From normal complement serum	...	n. end-piece and n. mid-piece.
" thermoinactive complement serum (5 mins. 55°)	end-piece II and mid-piece II.	
" " " (30 mins. 55°)	end-piece III and mid-piece III.	

In the following haemolysing tests always 1 c.c. sens. red corpuscles is taken, the AB dose being 0.0025 c.c.

							Haemolysis
1.0 c.c. thermoinactive serum (2 mins. 55°)	+ 1.0 c.c. 0.85 % NaCl	
1.0 c.c. " " (3 mins. 55°)	+ 1.0 c.c. " "	
1.0 c.c. " " (5 mins. 55°)	+ 1.0 c.c. " "	
1.0 c.c. " " (30 mins. 55°)	+ 1.0 c.c. " "	
1.0 c.c. thermoinactive serum (2 mins. 55°)	+ 1.0 c.c. $\frac{1}{10}$ active complement	
1.0 c.c. " " (3 mins. 55°)	+ 1.0 c.c. " "	
1.0 c.c. " " (5 mins. 55°)	+ 1.0 c.c. " "	
1.0 c.c. " " (30 mins. 55°)	+ 1.0 c.c. " "	
1.0 c.c. n. end-piece + 1.0 c.c. 0.85 % NaCl	
1.0 c.c. n. mid-piece + 1.0 c.c. " "	
1.0 c.c. n. end-piece + 1.0 c.c. n. mid-piece	
1.0 c.c. end-piece II + 1.0 c.c. 0.85 % NaCl	
1.0 c.c. mid-piece II + 1.0 c.c. " "	
1.0 c.c. end-piece II + 1.0 c.c. mid-piece II	
1.0 c.c. end-piece III + 1.0 c.c. 0.85 % NaCl	
1.0 c.c. mid-piece III + 1.0 c.c. " "	
1.0 c.c. end-piece III + 1.0 c.c. mid-piece III	

1.0 c.c. n. end-piece + 1.0 c.c. mid-piece II	■
1.0 c.c. n. end-piece + 1.0 c.c. mid-piece III	□
1.0 c.c. n. mid-piece + 1.0 c.c. end-piece II	□
1.0 c.c. n. mid-piece + 1.0 c.c. end-piece III	□
1.0 c.c. thermoinactive serum (2 mins. 55°) + 1.0 c.c. n. end-piece	■
1.0 c.c. „ „ (3 mins. 55°) + 1.0 c.c. „	■
1.0 c.c. „ „ (5 mins. 55°) + 1.0 c.c. „	■
1.0 c.c. „ „ (5 mins. 55°) + 1.0 c.c. end-piece II	■
1.0 c.c. „ „ (5 mins. 55°) + 1.0 c.c. end-piece III	□
1.0 c.c. „ „ (30 mins. 55°) + 1.0 c.c. n. end-piece	□
1.0 c.c. „ „ (30 mins. 55°) + 1.0 c.c. end-piece II	□
1.0 c.c. „ „ (30 mins. 55°) + 1.0 c.c. end-piece III	□
1.0 c.c. thermoinactive serum (2 mins. 55°) + 1.0 c.c. n. mid-piece	□
1.0 c.c. „ „ (3 mins. 55°) + 1.0 c.c. „	□
1.0 c.c. „ „ (5 mins. 55°) + 1.0 c.c. „	□
1.0 c.c. „ „ (30 mins. 55°) + 1.0 c.c. „	□

Analysing these experiments one may conclude:

Thermoinactive serum can be reactivated by adding fresh active serum (cf. H. Schmidt, 1913). The quantity of active serum necessary to produce an haemolytic effect on sensitized red cells in combination with the thermoinactive serum depends upon the anticomplementary property of the latter, which is greater the longer the serum is exposed to 56°. The reactivation of complement action by the isolated fractions depends also upon the intensity of the exposure to heat. In thermo-inactivation first the end-piece and then the mid-piece is destroyed (Sachs, 1913), and in guinea-pig serum inactivated by the usual way (30 minutes, 56°) no mid-piece is to be traced (Husler (1912)). In agreement with this statement guinea-pig serum only a short time—5 minutes—exposed to heat, long enough to render the serum inactive, can be reactivated by splitting it and mixing the two fractions again, as was also found by Mutermilch (1911). But this phenomenon is not obtained if the time, during which the serum is heated, has been longer. If the treatment by heat is too long, say 30 mins. or longer, it loses its property of being reactivated by end-piece, but the thermostability of the complement fractions seems to vary in different guinea-pigs.

Reactivation of shaken serum by thermoinactive serum.

With regard to the reactivation of shaken inactive serum by thermoinactive serum I give the following experiments:

Exp. XV. The following haemolysing tests were made with 1.0 c.c. sensit. red cells, the AB dose being 0.0025 c.c.

							Haemolysis
1.0 c.c. shaken inactive serum	+	1.0 c.c. $\frac{1}{10}$ complement	■
1.0 c.c. "	"	" + 1.0 c.c. n. end-piece	■
1.0 c.c. "	"	" + 1.0 c.c. thermoinactive ser. (2 mins. 55°)	■
1.0 c.c. "	"	" + 1.0 c.c. " (3 mins. 55°)	■
1.0 c.c. "	"	" + 1.0 c.c. " (5 mins. 55°)	■
1.0 c.c. "	"	" + 1.0 c.c. " (30 mins. 55°)	□
1.0 c.c. control serum		+ 1.0 c.c. " (2 mins. 55°)	■
1.0 c.c. "	"	" + 1.0 c.c. " (3 mins. 55°)	■
1.0 c.c. "	"	" + 1.0 c.c. " (5 mins. 55°)	□
1.0 c.c. "	"	" + 1.0 c.c. " (30 mins. 55°)	□
1.0 c.c. shaken inactive serum	+	1.0 c.c. " (2 mins. 55°) 2 hrs. shaken	■
1.0 c.c. "	"	" + 1.0 c.c. " (5 mins. 55°)	□
1.0 c.c. "	"	" + 1.0 c.c. " (30 mins. 55°)	□
1.0 c.c. "	"	" + 1.0 c.c. NaCl	□

From this experiment it appears that shaken inactive serum can be activated by thermoinactive serum but only if the latter has not been exposed to heat for too long a time, so there is still some end-piece left active. I am inclined to render the activation by thermoinactive serum equal to that obtained by normal end-piece. Kashiwabara (1913) did not always succeed in obtaining the reactivation by thermoinactive serum probably because the time he used for the thermoinactivation had been too long, so that the end-piece was destroyed completely. I saw in some experiments a reactivation of shaken inactive serum produced by serum which had been heated half an hour to 56°, but here neither the reactivation nor the inactivity by shaking had been complete. Finally I was able to confirm the statement of Kashiwabara (1913) that thermoinactive serum loses the property to reactivate shaken inactive serum, if it has been shaken before. In shaking thermoinactive serum

the observation can be made that much longer time is required to produce mechanical coagulation. It may be that this fact stands in some relation to the statement of Mutermilch (1911), viz. that it takes a much longer time to precipitate the serum globulin by dialysis in heated sera than in normal sera.

Possible explanation of the inactivation by shaking.

Now is it possible to give a reasonable explanation of the nature of the shaking inactivation? Presuming the complement to be of the nature of a ferment the shaking inactivation of complement-containing serum can be placed in some relation with the inactivation by shaking of pepsin and trypsin (Shaklee and Meltzer, 1909) and of the lab-ferment (Schmidt-Nielsen, 1909). P. Schmidt (1911, 1912) showed that active complement is rendered inactive, if it is kept in contact with an emulsion of globulin, prepared from a thermoinactive serum, during 25-30 minutes at 37°. He supposes an adsorption, which increases with the time. Further, he showed that only an overplus of immune bodies, containing albumin, is able to set free the complement from the globulin surface. It is further shown by Landsteiner and Stankovic (1906) that coagulated serum protein is able to adsorb complement from a serum, thus rendering the serum inactive. In agreement with these facts it may be possible, that the coagulated serum proteins produced by the agitation adsorb the complement and that this adsorption increases with the time, thus explaining the observations of Ritz that a serum shaken for a long time loses its property of being reactivated by end-piece.

However, I was unable to obtain a loss of complement action when I brought fresh $\frac{1}{10}$ diluted complement-containing serum in contact with the precipitate, but this negative result may be due to an alteration of the degree of dispersity occurring in shaken sera.

In any way, the fact would be difficult to explain, that the isolated precipitate can by no means be reactivated while the supernatant inactive fluid can be reactivated by end-piece, if the complement were bound to the separated precipitate.

In view of these difficulties I must confess to be unable to give any satisfactory explanation of what takes place in the process of inactivation by shaking.

GENERAL CONCLUSIONS.

1. Guinea-pig serum can be inactivated by shaking, the process being generally the quicker the higher the temperature.

2. It is advantageous for the purpose of inactivation by shaking to dilute the serum to 1:10 with saline solution and to take as small a quantity in comparison with the volume, in which it is to be shaken, as the resultant froth formation will permit.

3. In every serum a coagulation of a part of the proteins occurs owing to the agitation.

4. This precipitate always precedes the inactivation in time but no direct connection exists between the constituents of the precipitate and the complement.

5. From the observations, viz. that oxygen does not influence either the effect of heat on complement or the period of its survival in storage or the inactivation by mechanical agitation, it follows, that no oxidation occurs during inactivation by shaking but that the latter is independent of the nature of the gas.

6. The precipitate in shaken sera cannot be reactivated either by mid- or end-piece.

7. The supernatant fluid can be reactivated by fresh complement, by normal end-piece and by thermoinactive serum. Thermoinactive serum can however reactivate shaken inactive complement, only if it still contains active end-piece.

8. If thermoinactive serum is shaken it loses its property of reactivating shaken inactive serum.

9. No satisfactory explanation of the inactivation by shaking can be adduced.

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COMPLEMENT ACTION IN REGARD TO SURFACE TENSION.

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INTRODUCTION.

IN recent years immunity reactions have come to be regarded more particularly from the physico-chemical standpoint. Many of the phenomena occurring in immunity reactions are shown to be of the nature of colloidal chemical processes, and the laws which govern such processes have been found to hold, at least partially, for reactions occurring in sera, which belong to the class of colloidal protein solutions. Thus the application to immunity reactions, of such conceptions as viscosity and surface tension, has afforded a more reasonable explanation of these reactions. In the following paper special reference will be made to the surface tension, changes of which play an important part in all immunity reactions.

Traube (1908) showed, that if fresh guinea-pig serum was heated for half an hour at 55° , its surface tension was diminished. Thus the inactivation by heat caused the production of substances, which lower the surface tension. Addition of fresh serum had the effect of restoring the complement action and causing the return of the surface tension to its original value after 20 hours. Traube showed further, that if the serum is only heated to 40° – 50° the diminution of the surface tension is less marked and complete restoration may occur spontaneously. Also the ageing of a serum is associated with inactivation and a loss of surface energy. These bathotonic substances, produced in the serum by thermoinactivation, must be adsorbed by the surface of any second phase, which may be added. Thus in the case of red corpuscles, according to Traube, a thickening of their lipoid sheath would occur owing to the adsorption of such substances present in an inactive serum, the result being an increase in their resistance to the haemolytic influence of the anchored amboceptor. These substances act therefore like antihaemolysins by weakening the effect of specific haemolysins. Now, according to Traube, complements are substances which annul this antihaemolytic resistance by destroying the lipoid sheath of the red cells, thus rendering the action of an haemolytic amboceptor effective. Traube supposes the anticomplementary power of some active sera to be due to the presence of peptone, which is a strong bathotonic substance by itself. With regard to the objection that the addition of sera, which have stood for a long time, produces the same effect on thermoinactive sera as the addition of fresh active serum, the author mentioned, that a thermoinactive serum, even in a dilution of 1 in 30, had still a well-marked anticomplementary action, although the value of its surface tension was scarcely to be distinguished from that of an equally concentrated active serum. His conclusion is therefore, that the smallest amount of bathotonic substances, too small to be traced by a stalagmometer, may produce anticomplementary action.

Mario Segale (1911) confirmed by his experiments those of Traube in regard to the alteration of the surface tension occurring in thermoinactivation and its restoration to the original value after 20 hours by adding fresh active serum. By means of the ultramicroscope Segale was able to show the existence of micelli produced by the action of heat on serum, but further experiments showed that the alteration of the surface tension is not due to the existence of these micelli. He found also that the complementary function of a mixture of equal parts of active and thermoinactive serum was diminished to 50 %, although the value of the

surface tension was quite normal, and *vice versa*, after centrifugalising the micelli out of a thermoinactive serum and adding them to a fresh serum, he was able to obtain a lowering of surface tension without an alteration of the complementary function of the serum. He concludes therefore that the bathotonic substances, which were produced either by the action of heat or of age, have nothing to do with the complement action of the serum.

In the following paper I give a record of a series of experiments undertaken to elucidate the possibility of an association between the value of the surface tension of a serum and its complementary function.








Technique of experiments.

The value of the static surface tension of a liquid was measured by the number of drops produced by the same volume of liquid under equal conditions. The number of drops does not give an absolute measurement of the surface tension, which latter is to be expressed by dyn./cm., but the observed values of the number of drops can be compared with each other and with that of water, because the formation of drops is, under equal conditions, a function of the surface tension. The measurements were made by a special stalagmometer, designed by Traube and manufactured by C. Gerhardt, Bonn. For my experiments I used three of them, which gave different numbers of drops for distilled water at the temperature of 20° C. (57·62, 58·89, 57·2). In order to obtain a better comparison of the values obtained by these different stalagmometers, I calculated the number of drops, which would be produced by a normal stalagmometer giving 100 drops for a certain volume of distilled water at the temperature of 20° C. If D_w indicates the number of drops for distilled water and D_f the number of drops found for the liquid in question, I give in the following experiments the values of D as calculated by the equation

$$D = \frac{100}{D_w} \cdot D_f.$$

Only the haemolytic complement of guinea-pig serum has been examined. Its complementary function was tested by its haemolytic power in combination with sensitized, three times washed sheep red corpuscles of which a 5% emulsion in 0·85% saline solution was used. The amboceptor was inactivated rabbit serum, the single lysing dose of which was 0·00125 c.c. by using 1 c.c. of the red cells emulsion and 0·1 c.c. of the complement-containing serum. In the following haemolytic tests

always the double amount of the above mentioned amboceptor dose was taken. To illustrate the different degrees of haemolysis the following scheme is used:

No haemolysis	
Trace	„	...	
Slight	„	...	
Half haemolysed	
Strong haemolysis	
Almost complete haemolysis			
Complete haemolysis	...		

Alteration of the surface tension by storage.

First of all I was able to confirm the observations made by Traube and Segale that in complement-containing sera, which have been kept for some time, an alteration of the surface tension takes place. This alteration occurs sooner the higher the temperature at which the serum is kept, just as the complementary power of serum disappears under similar conditions of time and temperature. But the alteration of the surface tension is relatively small and never reaches values, which correspond to those obtained by the action of heat (55° – 56°), and further the inactivation occurring in old complement-containing sera is in most cases not complete, for it is possible to observe a more or less strong complementary action occasionally even after the serum has been kept for weeks, if the complement dose is 0.1 c.c. or higher and strongly sensitized red corpuscles are taken. K. Hara (1913) lays special stress on the bacterial growth as causing the loss of complementary power and the formation of anticomplementary properties, which some sera develop if kept for a long time. How far the bacterial growth in old sera is responsible for the lowering of the surface tension is still a question for experiment. In any case, according to Hara, serum, if kept under sterile conditions, preserves its complement action much longer than sera for which sterility has not been observed. I found that neither freezing nor thawing altered the surface tension more than may be due to the time during which the serum is kept under these conditions. It is well known that low temperature has a preserving influence on immune bodies as well as on complement, and Ito (1912) was recently able to show that even the temperature of liquid air did not destroy the complementary function of a serum. This author found that, if serum is

kept a long time at a low temperature, it is disposed in layers such that the layer of serum at the bottom of the tube has a much stronger complementary action than the supernatant fluid. It would be interesting to know how far this phenomenon is due to surface energies.

Alteration of surface tension by dilution.

In most serological experiments the complement-containing serum is employed in a dilution of 1 : 10 in 0·85 % saline solution. Saline solution increases the surface tension of pure water in a slight degree, the "surface tension-concentration" curve being a straight line (Freundlich, 1909, p. 60). For an 0·85 % saline solution I obtained $D = 99\cdot59$. The surface tension of a serum increases with its dilution in saline solution, but here the "surface tension-concentration" curve does not follow a straight line, but is represented by a parabolic curve (Iscovesco, 1911 *a*) of which I give the figures in the following table :

EXP. I. *Alteration of the surface tension of fresh guinea-pig serum effected by dilution in 0·85 % saline solution.*

Concentration	Serum I <i>D</i>	Serum II <i>D</i>
1 : 1	110·98	110·37
1 : 1·25	—	108·76
1 : 1·5	—	107·92
1 : 2	107·75	106·78
1 : 3	106·53	105·58
1 : 4	106·09	104·94
1 : 5	—	103·99
1 : 7	105·61	—
1 : 8	—	103·55
1 : 10	105·05	103·07
1 : 20	104·88	102·07
1 : 40	103·91	101·24
1 : 80	102·52	100·72
1 : 320	100·54	—

For comparison I give in the following table figures representing the number of drops which have been obtained by the dilution of peptone with distilled water.

These figures if plotted give a curve of a similar type. According to Freundlich (1909, p. 65) such a curve can be represented in a first approximation by a general parabolic equation of the form

$$S_m - S_l = K \cdot C^n$$

in which K and n indicate constants, S_m the surface tension of the pure solvent and S_l that of the solution. The value of the surface tension can be calculated from the number of drops by a proceeding mentioned by Traube (1904 *b*). From these figures it may be concluded, that a fresh serum itself contains bathotonic substances (protein? salts of fatty acids? free gallic acid?), the tendency of which to lower the surface tension must decrease with their dilution.

EXP. II. *Relation between the surface tension and the concentration of peptone.*

c.c. of a 5% peptone soln. in aq. dist. added to 20 c.c. of distilled water	Resulting concentration of the peptone soln. %	D
0	0	100.0
0.05	0.0125	103.62
0.05	0.024	109.79
0.05	0.037	112.15
0.1	0.061	115.33
0.1	0.086	118.67
0.1	0.110	118.88
0.2	0.157	120.61
0.2	0.203	120.74
0.5	0.316	123.72
1.0	0.526	125.48
2.0	0.893	128.28
2.5	1.27	130.29

Alteration of surface tension by temperature.

Further I was able to confirm the observations of Traube and Segale, that a serum, if heated to 56° for half an hour, has its surface tension diminished by a considerable amount. If for instance the number of drops given by an active serum diluted in 1:10 by saline solution is 102.25, the same serum after being heated for half an hour to 55° – 56° gives 107.29 drops. The surface tension of an undiluted active serum giving 110.73 drops, is so diminished by the effect of heating at 55° for half an hour, that 114.24 drops are obtained. If such a heated, undiluted serum is diluted by saline solution its surface tension will increase, following the same curve as mentioned above, and from the following figures it can be demonstrated, that the number of drops belonging to a concentration of 1:20, does not differ from that corresponding to the same concentration of an active serum.

EXP. III. *Influence of dilution on the surface tension of active and thermoinactive serum.*







Concentration	D of active serum	D of inactive serum 30 mins. 55°
1 : 1	110·73	114·24
2	106·63	109·37
3	105·24	107·48
4	103·97	106·49
5	103·69	106·19
6	103·46	105·35
7	103·27	—
8	103·07	104·57
10	103·02	104·50
20	102·08	102·72
40	101·41	101·64
80	100·80	101·05

Traube (1908) has already mentioned these facts and said that the difference in complementary power is due to such a small amount of bathotonic substances, as cannot be measured by an ordinary stalagmometer. I think it is evident that in this case the difference in the complement action has no association whatever with the surface tension of the serum. If a concentrated fresh active serum gives about the same number of drops as the same serum gives if diluted to 1:2 and inactivated by 30 minutes exposure to 55°, it is difficult to construct an association between surface tension and complementary function of the serum.

Now the loss of surface tension due to the effect of a temperature of about 55° takes place much sooner than in half an hour, as can be shown in the following experiments.

EXP. IV. *Alteration of surface tension in relation to time of exposure at 55°.*

Undiluted complement-containing serum was exposed to 55° for varying times. It was then diluted 1 in 10 with saline solution and tested with regard to surface tension and complement action. (1·0 c.c. of the diluted serum in contact with 1 c.c. of the sensitized red cell emulsion.)

Time of exposure to heat	Haemolysis	D
0 mins.		102·24
6		105·01
12		105·12
18		104·96
24		105·07
30		104·98

The result shows that the value of the surface tension which is obtained by heating the serum to 55° is nearly reached in the same time in which the complement action of the serum is destroyed. (However after that short time the complement action can be restored either by adding fresh end-piece or by splitting the complement into the two fractions and mixing these together again, but after the influence of heat for half an hour at 55° C. such restoration is no longer possible, cf. H. Schmidt, 1913.) But if a serum which has been heated at 55° C. is subjected to the influence of a higher temperature, a further fall of surface tension occurs. A 1:10 diluted serum becomes an opalescent fluid if heated in boiling water and the colloidal state of the denaturated protein is of a very stable nature. The alteration of the surface tension in such a serum may be shown by the following figures:

EXP. V. *Alteration of the surface tension of a 1:10 diluted active serum at the temperature of boiling water.*

	D
1:10 diluted serum active	103.13
Do. after half an hour's exposure to 55° C.	108.93
Do. after heating in boiling water	112.04

I found in almost every serum which I examined, that the value of its surface tension, if it is heated only during 6 minutes to 55°–56°, is nearly the same as that which is reached by the effect of the same temperature in half an hour. Also the same short period of 5–6 minutes was sufficient to inactivate the serum, and the following experiment shows that the concentration under which the serum is heated does not influence the time necessary for the thermoinactivation.

EXP. VI. *Concentration of the serum in regard to the time of thermoinactivation.*

1.0 c.c. of fresh serum was exposed to 56° in different concentrations varying between 1:1 and 1:10 during different periods varying from 0 to 5 minutes. The serum was then diluted to 1:10 with saline solution and its complementary power tested. The result was that after three minutes no haemolysis occurred in any case, the control however giving good haemolysis.

The same astonishingly short time was found sufficient to render a serum inactive by heat in the recent experiments of Husler (1912). With regard to a paper of Noguchi and Bronfenbrenner (1911), however, who mentioned that sera treated in such a way are not completely

inactivated and that it is possible by using much larger doses than the usual 0.1 c.c. to demonstrate that the exposure of the serum to 55° even after half an hour is not sufficient to render it completely inactive, I give in the following the record of an experiment.

EXP. VII. *Influence of time in thermoinactivation.*

Haemolysis of 1.0 c.c. sensitized red cells by

	c.c....	1.0	0.5	0.25	0.15	0.1	0.0
I: 10 serum active	■	■	■	■	■	□
I: 10 serum treated 5 mins. at 55°		□	□	□	□	□	□
I: 10 serum treated 30 mins. at 55°		□	□	□	□	□	□
I. 0.15 c.c. 1:10 active serum		■	
II. Of the thermoinactive serum (5 mins. at 55°)							
0.15 c.c. 1:10		Same dose as in I ...				□	
0.15 c.c. 1:5		Twice the dose in I ...				□	
0.15 c.c. 1:10		10 times the dose in I				□	
0.3 c.c. 1:1		20 times the dose in I				□	
0.6 c.c. 1:1		40 times the dose in I				□	
0.9 c.c. 1:1		60 times the dose in I				□	
III. Of the thermoinactive serum (30 mins. at 55°)							
0.15 c.c. 1:1		10 times the dose in I				□	
1.5 c.c. 1:1		100 times the dose in I				□	

From these figures I conclude that at least in the case of guinea-pig serum the thermoinactivation has been complete after an exposure of 5 minutes to 55°, but these data are probably not transferable to the sera of other animals.











































Surface tension in regard to reactivation of thermoinactive serum.

By adding fresh serum to a thermoinactive serum a complete haemolytic effect can be obtained. The amount of fresh serum necessary for this effect depends upon the anticomplementary action of the thermoinactive serum, which may occasionally be so great, that the haemolytic action finally obtained by adding fresh serum, is merely due to the absolute amount of the latter, so that one cannot speak of a real restoration of the complementary action of thermoinactive serum.

I have been able to confirm the experiments of Traube and Segale, that after the loss produced by the heat, the surface tension reaches its normal level again after 24 hours by adding fresh active serum. But in order to recognise how far the haemolytic action produced in thermoinactive serum by addition of fresh serum agrees with the alteration of the surface tension I instituted certain experiments, of which the following may be quoted:

EXP. VIII, 1. *Alteration of surface tension occurring in thermoinactive serum by adding fresh active serum, compared with the haemolytic effect thus produced.*









































1.0 c.c. sensitized red corpuscles plus

Of	Relative amount of active ser.	c.c.							D
		1.0	0.75	0.5	0.25	0.15	0.1	0.0	
I. 1:10 active serum ...	1:10								103.33
II. 1:10 thermoinactive ser. (30 mins. 56°)	0								106.29
III. 22.0 c.c. of II + 0.2 c.c. 1:1 active ser.	1:100								105.88
IV. III + 0.2 c.c. active ser.	1:51								106.23
V. IV + 0.3 c.c. „ „	1:26.7								105.94
VI. V + 0.4 c.c. „ „	1:15.5								106.10

After the addition of active serum in III-VI, 2 c.c. of the mixture were taken off each time for the haemolytic test.

EXP. VIII, 2. *Instead of undiluted active serum, a 1 in 10 dilution was taken, but otherwise no alteration of the technique took place.*

1.0 c.c. sensitized red corpuscles plus

Of	c.c....						D	Relative amount of active serum in the various mixtures
		1.0	0.5	0.25	0.15	0.0		
I. 1:10 active serum ...							102.89	1:10
II. 1:10 inactive ser. (30 mins. 56°)							105.85	0
III. 20.0 c.c. of II + 0.5 c.c. 1:10 active ser.							105.30	1:410
IV. III + 1.0 c.c. 1:10 act. ser.							105.33	1:130
V. IV + 2.0 c.c. „ „							105.36	1:55.7
VI. V + 3.0 c.c. „ „							105.08	1:31.4
VII. VI + 3.5 c.c. „ „							104.96	1:22
VIII. VII + 3.0 c.c. „ „							104.89	1:17.7

Setting aside the question whether the haemolytic effect obtained by the addition of fresh serum to thermoinactive serum, is entirely due to the action of the active serum, after the anticomplementary power of the thermoinactive serum has been neutralized, or to a genuine restoration of the complementary power of the inactive serum, the experiment VIII shows, that haemolytic action can be obtained before the surface tension is altered in any effective degree. The value of the surface tension approached the original value very closely after about 20 hours (but not in all cases), the small difference still existing in the following figures being probably due to the influence of age.

Exp. VIII.	$\frac{1}{10}$ active serum	103.33
	$\frac{1}{2}$ hour 56°	106.29
	2.2 c.c. inactive serum + 1.1 c.c. + act. ser.	...	106.10
	After about 24 hours	...	103.92

The bathotonic substances produced by the thermoinactivation are naturally in a relatively higher concentration on the free surface. If therefore a large separating funnel is filled with an inactive serum, the surface tension may be expected to increase, if the lower part of the liquid is run out from time to time so as to permit the formation of a new surface (*v.* Exp. IX)

Exp. IX.	$\frac{1}{10}$ inactive serum (30 mins. 56°)	107.88
	Lowest portion ...	106.05
	Mixed together again ..	108.02

Experiment IX shows that a procedure of this kind does not produce any very appreciable increase of the surface tension. I have not observed any restoration of the complementary power of the serum by this treatment.

*Alteration of surface tension in serum effected by addition of
peptone.*










The anticomplementary action of some sera (for instance in cases of uraemia) is believed by Traube (1908) to be due to the effect of small amounts of peptone. Peptone Witte lowers the surface tension of water considerably. As already mentioned (Exp. II), I found that the number of drops corresponding to a 1.27 % peptone solution in water was 130.29. If therefore the inactivity of a serum is in direct relationship with the low surface tension, it may be possible to render an active serum inactive by adding some peptone, the more so, because the loss of surface tension

produced by peptone is much greater than can be obtained by thermo-inactivation.











The following experiment shows the action of peptone on serum with regard to surface tension.

EXP. X. *Influence of peptone on surface tension and activity of serum.*

D of a 2 % peptone solution in 0.85 % NaCl solution = 134.59.






	Haemolysis after...	$\frac{1}{4}$ hr.	$\frac{1}{2}$ hr.	1 hr.
1 c.c. complement + 1 c.c. saline sol. + 2 c.c. sensitized red blood cells emulsion				
1 c.c. complement + 1 c.c. 2 % peptone + 2 c.c. sensitized red blood cells emulsion				
1 c.c. saline sol. + 1 c.c. 2 % peptone + 2 c.c. sensitized red blood cells emulsion				

In the following experiment instead of 0.85 % saline as diluent, peptone in a dilution of 2 % in saline solution was taken as dilution medium of the complement-containing serum.

c.c. of 1:10 complement...	1.0	0.5	0.25	0.15	0.0	<i>D</i>
1. 1 c.c. active complement + 9.0 c.c. 2 % peptone in 0.85 % saline solution + 1 c.c. sensitized red blood corpuscles						105.05
2. 1 c.c. active complement + 9.0 c.c. 0.85 % saline solution + 1 c.c. sensitized red corpuscles						134.05

In spite of the differences in the surface tension no difference in the action of the complement can be observed. In mixture No. 1 a 1.6 % peptone solution results, but nevertheless no anticomplementary action is observed.

In the following experiment instead of a 2 %, a 20 % peptone solution is taken.

20 % peptone solution in 0.85 % NaCl		0.85 % NaCl solution		1 % Compl. serum		Sens. red corpuscles	
0.2	+	0.8	+	1.0	+	1.0	
0.4	+	0.6	+	1.0	+	1.0	
0.6	+	0.4	+	1.0	+	1.0	
0	+	1.0	+	1.0	+	1.0	
0	+	0	+	0	+	3.0	

These strong peptone solutions show an anticomplementary effect.

1:10 active compl.		20 % peptone saline solution	<i>D</i>	
15 c.c.	+	0	102.93	} In all tubes <i>Haemolysis</i> complete, but a slight dimness remained.
15 c.c.	+	0.1 c.c.	117.97	
15 c.c.	+	0.2 c.c.	121.53	
15 c.c.	+	0.4 c.c.	123.75	
15 c.c.	+	0.8 c.c.	123.96	

Experiment X shows that it is impossible to render a serum inactive by adding some peptone and thus lowering the surface tension. A relatively large amount of peptone is required in order to exhibit anticomplementary action.

From the experiments above mentioned it is evident that the value of the surface tension does not permit one to form any conclusions with regard to the complement action.

Surface tension with regard to inactivation by adsorption.

The following experiments deal with the behaviour of the surface tension in response to certain other procedures which deprive a serum of its complementary action. Many authors (see Sachs, p. 870) have shown the possibility of removing the complement by digestion with suspensions of organic cells or inorganic substances. The adsorption of the complement by kaolin is especially strong, as the experiments of Laudsteiner and Stankovic (1906) have shown, the results of which are confirmed by Friedberger and Salecker (1911), who found that a contact of 2.0 c.c. normal guinea-pig serum with 0.2 c.c. kaolin for half an hour is sufficient to adsorb the complement completely. After centrifugalising a serum which has been treated with kaolin the supernatant fluid is found to contain a poisonous substance, which, if intravenously injected, is able to kill guinea-pigs in a very short time (Mutermilch, 1913). There is still some dispute as to the identity of this substance with the so-called anaphylatoxin. Friedberger, Salecker and also Mutermilch found that kaolin did not produce this poison, if the serum has been previously inactivated by heat at 56°. Mutermilch showed further that the serum became the more poisonous for guinea-pigs the larger the amount of kaolin which was employed to remove the complement, and he took the quantity of removed complement as a measure of the toxicity of the serum. By employing sulphate of barium (3 g. $\text{Ba}(\text{SO}_4)_2$ + 8 c.c. undiluted serum) as adsorbent, he observed neither loss of complement nor any formation of the presumed poisonous substance. The following experiment shows the alteration of the surface tension produced by kaolin in complement-containing serum.

These figures show, that the treatment by kaolin has produced in all three sera such an increase of the surface tension as to render the latter about equal to that of water, but the supernatant fluid in 1 and 2 was of a yellowish colour compared with the water-clear fluid in 3. Only the latter proved to be free of protein and no change occurred on

reheating, while by this procedure in the fluids (1) and (2) a well-marked fall of surface tension was observed and protein could be traced by boiling and adding acetic acid. Segale (1911), as already mentioned, found that the thermoinactivation of a serum was associated with a decrease of the degree of dispersion of the colloidal protein substances and the

EXP. XI. *Change of surface tension produced by kaolin.*

	<i>Before the treatment by kaolin D</i>	<i>After the treatment by kaolin D</i>	Centrifuged and then exposed for $\frac{1}{2}$ hour to	
			56° D	boiling temp. D
1. 1:10 diluted active serum	102.25	100.02	107.29	108.43
2. 1:10 diluted serum $\frac{1}{2}$ hr. exposed to 56°	108.16	101.02	—	107.99
3. 1:10 diluted serum boiled	112.04	100.42	100.42	100.5

same occurs on diluting a serum, according to P. Schmidt (1912). Now it is known (Sachs, 1913, p. 871) that the degree of dilution is an important factor in the adsorption of complement by any adsorbent, in the sense that the concentration is inversely proportional to the adsorption.

This dependence on concentration appears to be due probably to the lower degree of dispersity of the colloid in diluted sera, but the experimental data quoted above show that in thermoinactive serum as well as in active serum, kaolin does not remove all proteins. This effect took place only in the case of boiled serum, the degree of dispersity being then very small. Now the fact that in inactivated serum, there is still some protein left after the treatment by kaolin, renders the statement of Mutermilch and Friedberger, that such serum has lost its toxic property, the more interesting, especially if the surface tension is considered, which in both cases is rendered higher than that of the original serum. (Undiluted serum gives the same phenomenon, only the proportions of the figures being changed.) Friedberger (1911) found that the amboceptor is not removed from an immune serum by kaolin. This latter fact, taken in conjunction with the statement that kaolin removes all albumin, led him to suggest the possibility of proving the non-protein nature of the amboceptor. From my experiments, however, I think this suggestion to be very improbable.

In order to compare the adsorption of complement by kaolin with that obtained by $\text{Ba}(\text{SO}_4)_2$ or a suspension of red cells, the following experiment was undertaken.

EXP. XII. *Change of surface tension in inactivation by means of mechanical adsorption.*

1. 1 c.c. guinea-pig serum + 9 c.c. 0.85 % sal. sol. + 0.7 g. $\text{Ba}(\text{SO}_4)_2$.
2. 1 c.c. " " + 9 c.c. " " + 0.7 g. kaolin.
3. 1 c.c. " " + 9 c.c. of a 7 % red cell emulsion.
4. 1 c.c. " " + 9 c.c. 0.85 % sal. sol. ($D=103.18$.)

$\text{Ba}(\text{SO}_4)_2$ and kaolin were previously purified and neutralized.

These mixtures were kept at 37° for 1 hour and shaken from time to time, then centrifuged and the supernatant fluid tested with 1 c.c. sensitized red corpuscles, all tubes being filled up to 2 c.c.

c.c....	1.0	0.75	0.5	0.25	0.15	0.0	D
1. $\text{Ba}(\text{SO}_4)_2$							102.79
2. Kaolin							101.73
3. Blood cells							103.69
4. Control							104.96

The experiment shows that only in the case of kaolin is the serum completely inactivated and the highest value of surface tension reached. While the power of adsorbing complement is common to most cell suspensions, the red corpuscles form an exception, only their stromata being able to adsorb complement (Sachs, 1913). The relatively low surface tension, which is observed in the case of the red cells, is probably due to the slight amount of haemolysis, which occurred in the mixture and which causes lowering of surface tension (Iscovesco, 1911 *b*).

I mention in this connection that I found neither mid- nor end-piece able to reactivate the supernatant fluid of a kaolin-treated serum and further that this fluid obtained by the treatment either of an active or of an inactive serum by kaolin has no anticomplementary action, if added to a haemolytic system.

Surface tension in regard to inactivation by mechanical agitation.

























A further method of inactivating a serum is the treatment by means of mechanical agitation, a method inaugurated and known by the experiments of Jakoby and Schnetze (1910). Reference is made to this method in a special paper (H. Schmidt, 1913) and therefore I refrain from referring to the many recent works devoted to this subject, and I give here in this connection only a record of my experiences in connection with the inactivating of complement by mechanical agitation in so far as surface tension is concerned.

If a serum is shaken a more or less stable froth is formed. The formation of a froth is due to two circumstances: first of all, to the presence of a substance, which lowers the surface tension of the water and accordingly has the tendency to aggregate in the free surface, and secondly to the effect of surface viscosity, such as occurs in the case of bathotonic substances forming membranes on the surface (like peptone for instance). (Metcalf (1905), Freundlich (1909), p. 303, Ramsden (1804, 1904).)

If therefore water containing bathotonic substances, which can produce membranes on the surface, is shaken in air, the free surface of the liquid against air is enormously enlarged by the froth thus formed and a relatively larger amount of the bathotonic substances are to be found in the froth than in the liquid. By removing the froth and repeatedly shaking the liquid several times it may be possible to extract the bathotonic substances out of the liquid and to concentrate them in the centrifugalised froth. On this point I give the following experimental data obtained with complement-containing serum.

EXP. XIII. *The surface tension and complement activity of the froth compared with those of the remaining fluid.*

Fresh active complement-containing serum diluted 1:10 is shaken and from time to time the froth removed and centrifugalised. Afterwards both, froth and serum, were tested by the stalagmometer and with 1 c.c. sensitized red corpuscles in regard to their complement action, each tube being filled up to 2 c.c.

	<i>D</i>	c.c. 1·0	0·5	0·25	0·15	0·1	0·0
1. 1:10 active serum	102·85						
2. Froth	103·81						
3. Remaining fluid	102·57						
4. Mixture of 2 and 3 (equal parts)	102·67						

This experiment XIII shows that the froth contains a larger amount of bathotonic substances than the remaining liquid, but although I made a series of various experiments I never succeeded in detecting the complement either in the froth or in the residual liquid. The collected and centrifugalised froth had in all my experiments the same complementing power as the residual fluid. With regard to surface tension however it can be shown that an experimental error occurs, if for the measurement of the surface tension the froth is not taken into account.

I give in the following a series of experiments with regard to the alteration of surface tension occurring in sera which have been inactivated by shaking them at 36°.

EXP. XIV. *Alteration of surface tension in shaken sera.*






I. $\frac{1}{10}$ diluted serum was shaken 4 hours at 36°. As control the same serum in equal concentration was kept for the same time at 36°.

Haemolysis was tested with 1 c.c. sensitized red corpuscles.

	c.c. of $\frac{1}{10}$ compl. sol....	1.0	0.75	0.5	0.25	0.15	0.1	0	D
Untreated serum ...		■	■	■	■	□	□	□	101.79
Control serum ...		■	■	■	■	□	□	□	103.85
Shaken serum ...		□	□	□	□	□	□	□	103.57
After shaking, heated $\frac{1}{2}$ hour to 56°		□	□	□	□	□	□	□	110.37

II. $\frac{1}{10}$ diluted serum was shaken in equal tubes of 20 c.c. volume but in different quantity, thus varying the intensity of agitation. Time of shaking was $5\frac{1}{2}$ hours at 36°.

Haemolysis was tested with 1 c.c. sensitized red corpuscles.

		Tubes containing serum in amounts of				
		Untreated serum	Control serum	I 15 c.c.	II 10 c.c.	III 8 c.c.
D (stalagmometer)	...	101.90	102.57	102.36	102.22	102.31
Haemolysis of 1 c.c. compl.						

III. Equal quantities of complement-containing serum was placed in equal tubes of 20 c.c. volume shaken in different concentration, at 36° during 4 hours.

	Untreated serum $\frac{1}{10}$	Control serum $\frac{1}{10}$	I $\frac{1}{2}$	II $\frac{1}{4}$	III $\frac{1}{8}$	IV $\frac{1}{16}$
Concentration						
D, before shaking	103.18	103.18	107.41	105.00	103.88	103.18
D, after shaking	—	104.31	106.76	104.21	103.48	104.26

Haemolysis of 1 c.c. sensitized red corpuscles produced by complement, each tube filled up to 2 c.c.

	Complement in c.c.	0.1	0.05	0.025	0
Control serum ...		■	■	■	□
Concentration $\frac{1}{2}$ (I)		■	■	■	□
Do. $\frac{1}{4}$ (II) ...		■	■	■	□
Do. $\frac{1}{8}$ (III) ..		■	■	■	□
Do. $\frac{1}{16}$ (IV) ...		■	■	■	□

From these experiments one may conclude, that the surface tension of a shaken inactivated serum lowered, but generally this loss of surface energy does not differ much from that occurring in the control serum

and is at least partly due to the effect of the temperature of 36°. If the serum is shaken at room temperature (16°) the loss of surface tension which can be observed is very small, but here it is difficult to get the serum completely inactive. To obtain the following data a serum has been shaken at 16° during 6 hours.

EXP. XV.	<i>D</i>
$\frac{1}{10}$ diluted serum ...	102.58
Shaken 6 hours at 16°	103.10
„ 5 hours at 37°	103.41
Control serum „	103.2

If the serum has been inactivated before by heating it to 56° for half an hour and also by boiling it, the following experiment shows that such sera do not suffer an alteration of their surface tension, if shaken a long time either at room temperature or at 37°.

EXP. XVI.	<i>D</i>
$\frac{1}{10}$ diluted serum active	102.58
„ „ inactive (30 mins. 56°) ...	107.45
„ „ „ 6 hours shaken at 16°	107.57
„ „ „ 5 „ „ 37°	107.86
Control serum at 37°	107.34
$\frac{1}{10}$ diluted serum boiled	109.65
„ „ „ 6 hours shaken at 16°	109.56
„ „ „ 5 „ „ 37°	109.73
„ „ „ Control at 37° ...	109.48

If such a shaken and completely inactive serum is exposed to a temperature of 56° for about 5–10 minutes or longer, the surface tension will be promptly lowered. The same occurs if a shaken thermoinactive serum is brought to the temperature of boiling water.

The only author who took the surface tension into consideration in experiments undertaken to destroy complement by mechanical agitation was Bertolini (1911). This author shook sera 8–10½ hours at a temperature of 18–20°. He could not obtain a complete inactivation of the shaken sera, but he saw the formation of micelli, as Segale did in inactivated sera. After removing the micelli out of the shaken serum, he showed that the surface tension which was lowered during the agitation, increased to its original value. He concludes that these alterations of the surface tension have nothing to do with the complement function of the serum. The micelli found by Bertolini are very probably the same as Ramsden's coagula and not identical with Segale's micelli found in thermoinactivated sera. The coagulation by shaking is,

according to Ramsden (1894), different from that produced by the effect of high temperature, by the different behaviour of the coagulated protein against KOH and HCl. It takes a much longer time to get a coagulation in thermoinactive sera by shaking them, in fact, it is a phenomenon which I have very rarely observed, but in boiled sera I never saw any change occurring by mechanical agitation. This formation of coagula is always to be observed, before a serum is rendered inactive by shaking, but *vice versa* a serum cannot be considered as inactive when this coagulation has occurred. With regard to surface tension it is evident that the removal of a part of that substance, which lowers the surface tension, must be followed by an increase of the latter, and if this increase cannot be observed, it must have been annulled by other influences such as the temperature of 37° (the effect of which is indicated by the control), and also possibly by local increase of temperature due to inner friction in the shaken tube. Such local increase of temperature may be sufficiently effective to influence the surface tension but not detachable by an ordinary thermometer.

CONCLUSIONS.

1. By keeping fresh guinea-pig serum a long time a loss of surface tension occurs, but the loss is small and does not correspond with the inactivity, which is generally not complete. It is possible that bacterial growth influences the surface tension.

2. Dilution with saline solution increases the surface tension, whether the serum is active or inactive. An alteration of the surface tension in high dilution is scarcely to be detected.

3. Exposure to heat causes a well-marked lessening of the surface energy, and the time necessary to produce it is nearly the same as that required for inactivation by heat.

4. This time is about five minutes and does not alter with the concentration of the serum. In spite of the shortness of the time the serum proved to be completely inactive.

5. Exposure to the temperature of boiling water causes a further diminution of the surface tension.

6. The reactivation of thermoinactive serum by adding fresh serum takes place before the surface tension has altered in any effective degree.

7. Peptone Witte produces a well-marked fall of surface tension, if added to fresh serum in such amount that the serum action is not inhibited.

8. Kaolin if added to serum and centrifuged increases the surface tension of the serum, active serum being rendered completely inactive. In active and in thermoinactive serum kaolin does not remove all protein, so that further exposure to heat lessens the surface-tension. Only in boiled sera is all protein removed. The supernatant fluid can not be reactivated by either fraction of the complement.

9. Sulphate of barium also produces an increase of the surface tension, but no inactivation of the serum, if employed in the same way as kaolin.

10. In the inactivation of a serum by means of mechanical agitation no fall of surface tension occurs; the observed slight loss is due to the effect of the temperature of 37°.

11. The froth produced by the shaking must be taken into account in estimating the surface tension, for it contains a relatively larger amount of bathotonic substance. But no attempt to obtain the complement in the froth when separated from the remaining fluid was successful.

GENERAL CONCLUSION.

From these facts one may conclude that the surface tension of a serum does not permit any inference to be drawn as to its complement-action. Further if any relation does exist between the surface energy and the complement-function, they are not directly associated.

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THE ISOLATION OF TYPHOID BACILLI FROM FAECES BY MEANS OF BRILLIANT GREEN IN FLUID MEDIUM¹.

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Western Infirmary, Glasgow.)*

Review of the principal methods of isolating typhoid bacilli from faeces.
The methods hitherto suggested for the cultivation of typhoid bacilli from faeces have not proved entirely satisfactory though they have been of service. The constitution of media intended to aid the isolation of *B. typhosus* has been arranged according to two principles, (a) the use of substances which enable colonies of typhoid bacilli to be distinguished by their naked-eye appearance, and (b) the introduction into the medium of some substance which is intended to favour specially the growth of *B. typhosus* as compared with the other organisms which are present. Many of the media in common use fulfil both of these conditions to a greater or less extent. Thus the exclusion of Gram-positive organisms is secured by the use of crystal-violet, as in the medium of Conradi-Drigalski. This dye has very little inhibitory action on bacilli of the colon-typhoid group, although it is one of the most powerful antiseptics known so far as Gram-positive organisms are concerned. The exclusion of cocci from cultures of faeces, however, does not afford great aid in the isolation of *B. typhosus*, since the chief difficulty lies in the presence of large numbers of bacilli of the coli-group. The further addition to a solid medium of a sugar which is fermented with the production of acid by coliform bacilli, but not by *B. typhosus* and, at the same time, the

¹ We have much pleasure in acknowledging our indebtedness to the Carnegie Trust for a grant toward the expenses of this work.

incorporation of an indicator, is accordingly the device most commonly employed, *e.g.* in the media of MacConkey, Conradi-Drigalski and Endo. Such media have two serious disadvantages: firstly, almost every viable organism belonging to the typhoid-coli group produces a colony; hence, if typhoid bacilli are present only in scanty numbers in the faeces, it will be necessary to make the inoculation with a very considerable quantity of the material in order to include *B. typhosus* at all. Further, in order to obtain discrete colonies a large surface of medium is required. In fact, the larger the amount of faeces examined and consequently the greater the surface of medium employed, the greater will be the percentage of positive results. Obviously such a procedure may take much time to carry out. The second disadvantage of such media is that while some typical *B. coli* can be recognised with certainty there are many organisms which produce colonies more or less similar to *B. typhosus*; so that the lengthy process of examining many individual colonies may have to be resorted to.

Malachite green (derivative of tetramethyl-diamido-triphenyl-methane) and brilliant green (the corresponding tetra-ethyl compound) are bodies which possess the property of inhibiting *B. coli* to a greater extent than *B. typhosus*. These substances are both mentioned by Conradi and Drigalski (1902); but this selective action of malachite green appears to have been first of all made practical use of by Löffler; it has been employed in this country by Savage in his extensive investigations on infections with Gaertner's bacillus. Lentz and Tietz employed malachite green in a concentration of 1:6000 in agar as a means of obtaining elective growth of *B. typhosus* from faeces. According to their procedure, if typhoid colonies are not readily found in a plate of 10 cm. diameter inoculated with 0.1–0.2 c.cm. of a one in three suspension of faeces after 20 hours incubation at 37° C., then an emulsion of the whole growth on the plate should be made in 2 c.cm. of fluid and a loopful of this emulsion should be used to inoculate a plate of Conradi-Drigalski medium. According to Gaechtgens and Brückner this is the most efficient method so far devised for the isolation of typhoid bacilli; but they remark that a further improvement in methods of isolating *B. typhosus* is desirable. Conradi (1908) introduced later a solid agar medium containing brilliant green and picric acid, which has been further modified by Fawcett. The fact that these dyes exert a selective bactericidal action on *B. coli* as compared with *B. typhosus* has not, however, been generally accepted (Kathe and Blasius, 1909). Löffler drew attention to the fact that it was possible to inhibit the growth of

B. coli in a mixture with *B. typhosus* when inoculated into fluid media containing malachite green, so that a pure culture of typhoid bacilli was obtained. Lentz and Tietz recognised that the employment of a fluid medium would present marked advantages for the isolation of *B. typhosus* from faeces; but their attempts to employ malachite green in this way resulted in failure, as the typhoid bacilli were overgrown by other organisms.

Observations on the action of brilliant green on organisms of the typhoid-coli group. In the course of systematic investigations on the antiseptic action of benzol-derivatives¹ we found that a marked difference existed between the action of dyes which are members of the diamido-triphenylmethane group and those of the triamidotriphenylmethane series. Both groups exert a powerful action on Gram-positive organisms, e.g. staphylococci, anthrax bacilli (the bactericidal action of gentian violet has recently been brought into prominence by Churchman and Michael). But the diamidotriphenylmethane group (malachite and brilliant green) are also fairly actively bactericidal toward the typhoid-coli group, whereas these organisms are comparatively insusceptible to the triamidotriphenylmethane dyes (hexamethyl and hexaethyl violet). On comparing malachite and brilliant green it was found that the latter was much the more active in its inhibitory effect on the coli-group.

The examination of 54 strains of *B. coli* of the types commonly found in faeces, which had been isolated from faeces, urine, appendix abscesses, etc., and comprising *B. coli* No. 71 (MacConkey), *B. coli communis* (Escherich), *B. neapolitanus*, *B. schafferi*, *B. vesiculosus*, Grünthal's bacillus, *B. coscoroba*, *B. coli* No. 106 (MacConkey)², showed that these organisms were without exception more susceptible to the bactericidal action of brilliant green than were any of the 21 strains of *B. typhosus* which we have investigated. *B. coli unaerogenes* (6 strains), and certain of the "paracolon" bacilli (bacilli which do not ferment lactose and do not form indole, but which differ from the paratyphoid group either in being non-motile or in not fermenting dulcitol and which probably do not possess specific pathogenic properties), also dysentery bacilli and *B. faecalis alcaligenes*, all belong to the susceptible group. More resistant than the above mentioned types, but less resistant than *B. typhosus*, is *B. coli* No. 74 (MacConkey). *B. proteus* (13 strains) is on the average

¹ We are indebted to Prof. Ehrlich and to Messrs Bayer, Cassella, and Lucius-Brüning for their kindness in placing specimens of dyes at our disposal.

² The classification of these organisms has been made according to MacConkey's criteria.

somewhat less resistant than *B. typhosus*, although some strains are of practically equal resistance. To the group of organisms which are more resistant to brilliant green than *B. typhosus*, belong the paratyphoid bacilli, Gaertner's bacillus and certain members of the coli-group which occur only in scanty numbers in faeces. The resistant coli-types include inosite-fermenters, e.g. *B. lactis aerogenes*, B. No. 67 (MacConkey) and also some non indole-forming types of *B. coli* (certain of which fermented lactose only after mutation, as described by Penfold).

*The fluid medium containing brilliant green employed for isolating
B. typhosus and B. paratyphosus.*

The results following the inoculation of a medium which contains substances actively inhibitory to bacterial growth depend to a very great extent on the number of organisms which are introduced, as Krumwiede and Pratt have also noted. A small number of organisms may be entirely killed off, whereas growth may follow the introduction of a large number. This holds good both in the case of solid and of fluid media; accordingly we have concluded that it is useless to expect that a standard medium suitable for all cases can be devised. A further consideration of importance is that in making inoculations with faeces a considerable amount of organic material is introduced into the medium and there is no convenient method of standardising this element in different cases. For this reason also it is impossible to set up any single medium of fixed constitution. Accordingly we have proceeded on the principle of adding *varying amounts* of brilliant green to a series of tubes of peptone water. Each tube is then inoculated with a large loopful of faeces. If the faeces are not naturally fluid, then several volumes of sterile 0.85 per cent. NaCl solution are added and an emulsion is made. After the brilliant green peptone water cultures have been incubated for 20–24 hours at 37° C. subcultures are made from each tube on plates containing some medium with an indicator, which are then examined for *B. typhosus* after they have been incubated for 18–24 hours at 37° C.

Method. The practical details of the method are as follows:—peptone-water is prepared in the usual fashion; 20 grams of Witte's (Rostock) peptone and 5 grams of NaCl are added to 1000 c.cm. of distilled water; the mixture is steamed in a Koch's steriliser for $\frac{5}{4}$ of an hour and filtered through paper: 5 c.cm. are then distributed in 6" x 5/8" test-tubes which are plugged with cotton-wool and sterilised

at 120° C. for 15 minutes in the autoclave (the medium reacts faintly alkaline to litmus). The stock-solution of brilliant green (Bayer's Brilliant Green Extra Cryst.) consists of 1 per cent. of the dye in distilled water; this is freshly made up every 2-3 weeks. Immediately before use a 1:10,000 dilution of the dye is prepared by adding 0.1 c.cm. of the stock-solution to 9.9 c.cm. of distilled water. Of this dilution the following amounts are added to successive tubes of the peptone-water, viz. 0.04, 0.08, 0.12, 0.16, 0.22, 0.3 c.cm. A loopful of faeces (up to 0.4 cm. diameter where the specimen is very fluid) is then at once added to each tube and the contents are well mixed. After 20-24 hours incubation at 37° C. a loopful of material is taken from each tube and successive strokes are made on plates of MacConkey's medium (two 10 cm. plates in all are quite sufficient to accommodate 3 strokes from each dilution). The plates are then incubated as usual and are examined for typhoid bacilli.

Using this method, we have isolated *B. typhosus* from 11 cases which were clinically typhoid fever and paratyphoid bacilli from other two. In order to obtain an estimate of the number of typhoid bacilli present as shown by ordinary methods, a plate of MacConkey's medium 8 cm. in diameter was at the same time inoculated directly from the faeces in each case by making successive strokes. In seven of the 13 cases it was impossible to detect the presence of typhoid or paratyphoid bacilli in the direct plates; in five of these *B. typhosus* was recovered in the brilliant green cultures and in the other two paratyphoid bacilli. In the remaining six cases typhoid bacilli were observed in the direct cultures. It is to be noted, however, that in every instance in which typhoid bacilli were obtained by direct culture they were also demonstrated in the brilliant green cultures. It is interesting to observe that frequently a particular concentration of brilliant green yielded a practically pure growth of *B. typhosus* where only scanty colonies were found in the plate inoculated directly with faeces¹. The advantage of making a series of cultures in medium containing different quantities of the dye is shown by the fact that there appears often to be an optimum concentration of dye favourable to the growth of the typhoid bacilli. This point is well illustrated by the following example:

¹ *B. typhosus* was identified in every instance by its culture reactions which were tested shortly after isolation (presence of motility in culture after six hours at 37° C.; fermentation of glucose and mannite without gas production, negative result with lactose, dulcitol, saccharose; no indole formation). In every instance the agglutination-test carried out subsequently with a powerful anti-typhoid serum confirmed the diagnosis based on the culture reactions.

Case A. M. Subcultures on MacConkey's medium from 24-hour brilliant green peptone water cultures of faeces.

Amount of brilliant green (1:10,000) present in the peptone water cultures (5 c.cm.)	Resulting growth
0.03 c.cm.	mainly red colonies
0.07 "	pure <i>B. typhosus</i>
0.11 "	mainly <i>B. typhosus</i> with an admixture of <i>E. proteus</i>
0.16 "	scanty colonies of <i>B. typhosus</i> , <i>B. proteus</i> and red colonies
0.22 "	ditto

(A subculture made after the 0.22 c.cm. brilliant green culture had been incubated for 36 hours at 37° C. yielded a pure culture of *B. typhosus*.)

The optimum concentration of brilliant green varies from case to case; thus contrast the following example with that quoted above:

Case O. R. Subcultures on MacConkey's medium from 24-hour brilliant green peptone water cultures of faeces.

Amount of brilliant green (1:10,000) present in the peptone water cultures (5 c.cm.)	Resulting growth
0.04 c.cm.	almost all red colonies
0.08 "	equal numbers of <i>B. typhosus</i> and red colonies
0.12 "	mainly <i>B. typhosus</i>
0.16 "	<i>B. typhosus</i> in pure culture
0.2 "	ditto
0.25 "	ditto

It is to be noted that we endeavoured in every instance to obtain the faeces as fresh as possible. This should always be secured when examining for typhoid bacilli, as faeces on standing will become free of typhoid bacilli even when these have been introduced artificially.

The isolation of *B. typhosus* from actual cases of disease is, of course, the true test of the efficacy of a method. At the same time it was of interest to ascertain how small a number of typhoid bacilli could be recovered from an artificial mixture with faeces. Thus, a fresh specimen of normal faeces was emulsified with two volumes of 0.85 NaCl solution and strained through wire gauze in order to secure a homogeneous emulsion. A loopful (diameter of the loop 0.4 cm.) of the emulsion was introduced into a series of 5 c.cm. peptone water tubes containing brilliant green (1:10,000), viz. 0.04, 0.09, 0.14, 0.19, 0.24, 0.3 c.cm.; at the same time a measured amount of a dilution of a 24-hour bouillon culture of a strain of *B. typhosus* of average resistance to brilliant green was

introduced. A similar amount of the dilution of the *B. typhosus* culture was plated on agar and yielded two colonies. The number of viable organisms present in the amount of faeces employed in each tube was 2800, as ascertained by plating on agar. Subcultures made from the brilliant green tubes after incubation at 37° C. for 24 hours gave the following results :

0.14 c.cm. brilliant green	numerous red colonies (? mixture of <i>B. typhosus</i> with the <i>B. coli</i>)
0.19 " " "	<i>B. typhosus</i> and red colonies in equal numbers
0.24 " " "	ditto
0.3 " " "	abundant colonies of <i>B. typhosus</i> and two red colonies

Thus, a practically pure culture of *B. typhosus* was obtained from a mixture of two typhoid bacilli along with 2800 other viable organisms. A control series of brilliant green cultures of the faeces, without the addition of *B. typhosus*, yielded nothing resembling typhoid bacilli. A direct inoculation of the faeces on MacConkey's medium gave a dense growth of *B. coli*. In a series of experiments in which a larger number of typhoid bacilli (10) were added to the same quantity of normal faeces, we failed to recover a typhoid growth by plating directly on MacConkey's medium, although practically pure growths of *B. typhosus* were obtained by means of brilliant green.

The number of cases which we have investigated is comparatively small; but the successful nature of our results has led us to publish this note in the hope that the method may commend itself to those who have greater opportunities for investigating cases of typhoid fever and who, therefore, are in need of a reliable procedure for the isolation of typhoid bacilli from faeces. We may also remark that this method enables one to isolate certain types of atypical *B. coli* which are occasionally present in small numbers in faeces, in virtue of their resistance to brilliant green. Such organisms, however, have not been found to interfere with the isolation of *B. typhosus* by overgrowing it.

Different samples of the same dye are known to vary somewhat owing to the fact that most commercial dye-stuffs contain a certain quantity of indifferent impurity; accordingly, we would suggest that before proceeding to employ a given specimen of dye, its action should be tested with a sample of faeces known to contain typhoid bacilli. Under all circumstances, where it is desired to isolate *B. typhosus* from faeces by this method, a series of five or six tubes containing different amounts of brilliant green should be inoculated. If larger quantities of

faeces are to be used for inoculation or if coliform bacilli are present in very great numbers, then correspondingly large amounts of peptone water and of dye will require to be employed.

SUMMARY.

(1) Brilliant green exerts an inhibitory effect on the growth of bacilli of the coli group commonly occurring in faeces, which is in general more marked than its action on *B. typhosus* and paratyphoid bacilli.

(2) By taking advantage of this property of brilliant green a method has been devised for isolating *B. typhosus* from faeces. The procedure adopted is the inoculation of a series of tubes of peptone-water medium containing varying amounts of brilliant green, incubating for 20-24 hours, and then the inoculation on a suitable solid medium from each tube.

(3) The reason for employing a series of concentrations of brilliant green is that the optimum concentration for the growth and isolation of *B. typhosus* varies from case to case, depending probably both on the proportion of typhoid bacilli present and on the number and character of the accompanying bacteria as well as on the organic faecal material.

(4) The method is very easily and rapidly carried out.

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GAERTNER GROUP BACILLI IN RATS AND MICE.

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OUR knowledge of the importance of the Gaertner group of bacilli in human and animal pathology has been slowly but steadily extending. Members of this group are etiologically associated with most of the cases of paratyphoid fever while the number of outbreaks of food poisoning shown to be due to these bacilli is rapidly increasing. In addition they have been found to be associated with a number of diseases in the lower animals.

The group may be divided into two sub-groups, one forming the true Gaertner organisms including *B. enteritidis*, *B. suispestifer*, *B. paratyphosus* β and possibly a few other strains, and the other consisting of closely allied but apparently non-pathogenic bacilli found in the healthy intestine (para-Gaertner bacilli).

The distribution in nature of this group of organisms is obviously of considerable importance and has been extensively studied during the last few years although previously almost entirely neglected. For a summary of the available data as to the distribution of these bacilli amongst man, animals, food products and in nature the Report by one of us to the Local Government Board (Savage 1913) may be consulted.

Briefly stated it may be said that when the closely allied para-Gaertner bacilli are differentiated, true Gaertner group bacilli are not present, or only found extremely rarely, in the healthy human intestine and with nearly equal rarity (apart from rats and mice) in the healthy animal intestine. Put another way, and excluding for the moment rats and mice, the available English data certainly negatives the view that these organisms are natural intestinal inhabitants of man or animals although such a distribution is frequently asserted to exist.

On the other hand while the evidence is against the view that true Gaertner bacilli are natural inhabitants of the healthy alimentary tract

of man and the domestic animals used for food it is now well known that this group of organisms is responsible for a good deal of disease in animals. Of such diseases may be mentioned some forms of septicaemia and other diseases of calves, pyaemic and septicaemic conditions in cows and other bovines, some cases of enteritis in cows, disease in parrots (psittacosis) and sparrows, and disease in rats and mice.

While it may be advanced with some confidence that the presence of members of the true Gaertner group in the domestic animals used for food is evidence of some relationship to a diseased condition or association with cases of disease due to these bacilli ("carriers") the evidence is not very definite as regards rats and mice. Some of the available evidence suggests that in these animals Gaertner group bacilli may be natural intestinal inhabitants and present as such, apart from any association, direct or indirect, with a diseased condition. Indeed Bainbridge (1912) in the Milroy Lectures for 1912 on paratyphoid fever and meat poisoning suggests, "It may well be that the rat's intestine is the true home of this organism (*i.e.* *B. enteritidis*), and that it reaches the alimentary canal of cattle and other domestic animals through the contamination of their food or bedding by rats."

As will be shown later the matter is not merely of scientific interest but is of real practical importance in relation to food poisoning outbreaks and other diseases due to this group of organisms.

There are a number of investigations which bear upon this question.

Uhlenhuth and Shern (quoted by Hübener 1910) frequently found Gaertner bacilli in the spleen of healthy tame rats and observed that rats inoculated intraperitoneally with rat serum often died from an outbreak of Gaertner enteritis.

Heuser (1910) examined about 100 mice and in 5 per cent. isolated Gaertner bacilli of both types (*i.e.* *B. enteritidis* and *B. paratyphosus vel B. suipestifer* according to German classification) from their intestinal contents. The mice showed no symptoms of disease. He also examined about 60 white rats and found *B. enteritidis* in about 5 per cent. but no hog-cholera bacilli (*B. suipestifer*). He found that by animal passage the bacilli of the hog-cholera group showed a distinct rise of virulence to rats.

Zwick and Weichel (1911) examined 177 mice and in 28 found Gaertner group bacilli.

Eckert (1909) examined a large number of samples of intestinal contents including those of five rats. He failed to find any Gaertner group bacilli in the rat excreta.

Evidence is also available in another direction. A number of observers have fed or infected mice and rats with material of different kinds and from these animals in a number of the cases Gaertner group bacilli have been recovered post-mortem. The material injected in many of the cases certainly did not contain Gaertner group bacilli as shown by its nature and from careful cultural investigations. The following may be mentioned.

Mühlens, Dahm and Fürst (1908) fed mice with different kinds of prepared food and a number of them died, Gaertner group bacilli being isolated. In all 74 died out of the 138 mice fed and from nearly all of these one or other member of the Gaertner group was isolated. They were all highly virulent. These bacilli could not be found in any of the foods by cultural examination.

Zwick and Weichel (1911) fed 140 white mice with 70 samples of salt meat and different forms of pig, all of which culturally examined failed to show Gaertner group bacilli. 85 (60·7 per cent.) of the mice died and from two of them *B. paratyphosus* β was isolated.

Somewhat similar results have been found by other observers. While these findings have usually occurred with rats and mice comparable results have been met with in guinea-pigs. For example Smallman (1905) injected over 200 guinea-pigs intraperitoneally or subcutaneously with cultures of *B. typhosus* either living or dead. In 22 instances (about 11 per cent.) organisms of the Gaertner group were obtained.

With these outbreaks must be associated the fact that spontaneous outbreaks of infectious disease amongst rats and mice are not uncommon. One of us has met with three definite outbreaks in his laboratory mice at widely separated intervals, two due to or associated with *B. enteritidis* and the third also due to a Gaertner group organism, the precise sub-group not being investigated.

The above facts suggest two separate possibilities. They may be taken as showing that Gaertner group bacilli are natural inhabitants of rats and mice, or they may be explained on the view that they are not really natural inhabitants, but when found are present either as "carriers" from contact with actual cases or are present after recovery from an infection with Gaertner bacilli not severe enough to be fatal.

The latter view seemed to us to be the more probable but to try and clear up the matter we have carried out an extended series of examinations of rats in this country.

Summary of investigations.

The work carried out consisted of the bacteriological examination of 41 rats obtained from different sources. All the rats were of the ordinary brown variety except two of the Cardiff animals, obtained from a ship from India, which were black rats.

Twenty-eight of the rats were obtained in Weston-super-Mare mostly killed on the refuse heaps of the town, the remaining few being killed in houses.

Ten of the rats were obtained from Cardiff through the kindness of Dr Walford, M.O.H. Seven of these were caught on board ships coming into Cardiff Dock, three were from houses in the city. The remaining three rats were from a small town in Somerset about 30 miles from Weston-super-Mare.

Methods of examination.

All the rats were as far as possible examined within a few hours of being received.

The same method of examination was followed throughout. The naked eye appearances of the organs were noted in each case.

Cultivations were made in the ordinary way from the spleen, liver and heart blood. The cultivations from the spleen and liver were into malachite green dultite broth, that from the heart blood into ordinary nutrient broth. If growth took place the liquid culture medium was plated upon neutral red lactose bile salt agar (L.B.A.) and the colonies identified. If there was no growth after two days at 37° C. the cultures were regarded as sterile.

In addition to these organs the intestinal contents were also examined. The examination was both direct and by enrichment. For the direct examination scrapings from both large and small intestine were added to sterile water in a test tube and an emulsion made. This emulsion was used to brush four L.B.A. plates in the ordinary way. After incubation for 24 hours at 37° C. the white colonies were picked off and subcultivated.

For the enrichment method loopfuls of intestine scrapings were added to tubes of dultite malachite green broth and incubated, usually for 24 hours, occasionally for 48 hours. If as was usually the case growth resulted this was plated on several L.B.A. plates and the white colonies investigated as for the direct plates.

The subcultivation method adopted for sorting out the possible true Gaertner group bacilli from the pseudo or para-Gaertner forms and non-Gaertner bacilli was to inoculate the white colonies into a compound sugar broth (C.S.B.). This was ordinary nutrient broth in double tubes containing 0.3 per cent. each of lactose, saccharose and salicin. True Gaertner bacilli do not ferment any of these substances so that if gas is produced the culture could be at once discarded as not a true Gaertner organism.

If after two days no gas production occurred the culture was subcultivated into glucose broth, litmus milk, peptone water, dulcete broth and upon gelatine slope. All organisms which reacted to these media like true Gaertner bacilli were then very fully worked out after being replated. Agglutination and pathogenicity tests were also employed.

The dulcete malachite green broth was used as an enrichment medium as in our experience it exerts, in the proportions used, a restraining action upon *B. coli* group organisms while encouraging Gaertner group bacilli to grow.

It may be added that the methods employed were all of proved usefulness and were methods which had been used very extensively by one of us (Savage, 1907-1910) for similar investigations of other materials.

For a number of the rats, in addition to cultural examinations, samples of blood from the heart were collected and serologically examined. The results are recorded below.

Results obtained.

It will serve no useful purpose to give the details of each individual bacteriological examination.

Rats obtained outside Weston-super-Mare. There were 13 rats in this group. No true Gaertner group bacilli could be isolated from any of them either from the liver, spleen or heart blood or from the intestinal contents. In a few instances pseudo-Gaertner strains were isolated.

Rats obtained from Weston-super-Mare. There were 28 in all in this group and somewhat different results were obtained. In 23 of these rats true Gaertner group bacilli could not be isolated either from the internal organs, heart blood or intestinal contents. From the remaining five rats true Gaertner group bacilli were isolated. The bacteriological findings in these five rats were as follows:—

Rat, No. 4. Examined April 24th, 1912. Obtained from town refuse heap. Numerous whitish pin-point areas on liver and scattered through the spleen.

Gaertner group bacilli isolated from spleen and liver (R_1 and R_2 respectively). Heart blood culture showed no growth. Although both the primary and secondary plates from the intestine showed white colonies none of these were Gaertner group bacilli.

Rat, No. 6. Examined April 24th, 1912. Same source as No. 4. One small apparently necrotic area on the liver, otherwise no naked eye abnormality. A Gaertner group bacillus isolated from the spleen (R_3). Cultures from heart blood and liver showed no growth. Numerous white colonies on both primary and secondary plates from the intestinal contents, but since all fermented the compound sugar medium or decomposed milk no Gaertner group bacilli were found.

Rat, No. 7. Examined April 25th, 1912. Same source as No. 4. No naked eye pathological appearances found. A Gaertner group bacillus (R_4) isolated from the spleen. Cultures from heart blood and liver showed no growth. The primary and secondary plates from the intestinal contents showed only a moderate number of white colonies. None of these were true Gaertner group bacilli but pseudo-Gaertner bacilli were fairly numerous.

Rat, No. 12. Examined May 20th, 1912. Obtained from a house. Rat had apparently died a day or so previously as organs were foul smelling. Apart from obviously putrefactive changes no abnormalities noticeable. Gaertner group bacilli isolated from the spleen and liver (R_5 and R_6 respectively). The heart blood culture showed growth but no Gaertner group bacilli were present as all slowly fermented the compound sugar medium. The plates from the intestinal contents both primary and secondary were all negative as regards the presence of true Gaertner group bacilli although pseudo-Gaertner bacilli were isolated.

Rat, No. 13. Examined May 20th, 1912. Found dying in a house and killed. No abnormalities noted. True Gaertner group bacillus (R_7) isolated from the spleen. Cultures from the liver and heart blood showed no growth. No Gaertner group bacilli could be isolated from the intestinal contents.

Summarising all the results it will be noted that true Gaertner bacilli were not isolated in any case from the heart blood or intestinal contents, but that in five rats true Gaertner group bacilli were isolated, from the spleen in each case, in two of these from the liver in addition.

The true Gaertner group bacilli isolated were culturally worked out and showed all the usual cultural characters including fermentation of glucose and dextrite, absence of fermentation of lactose, saccharose, salicin and glycerine, no indol production and acid followed by alkaline production in milk. They were all actively motile bacilli.

Their position in the Gaertner group (except the organisms isolated from rats 12 and 13), was determined by a series of agglutination tests. The Gaertner bacilli from rats 12 and 13 were obviously obtained from rats infected and ill from the Danysz rat virus which had been used and may be assumed to be that strain. The agglutination reactions of the others are shown in the following table.

Serum and dilution	R ₁	R ₂	R ₃	R ₄	<i>B. aertrycke</i>	<i>B. enteritidis</i>	<i>B. paratyphosus</i> β
<i>B. enteritidis</i> serum	1:100	+	+	+	+	+	
	1:1000	+	+	+	+	+	
	1:3000	+	+	+	+	+	
	1:5000	+ p.	+ p.	+ p.	+ p.	+ p.	
<i>B. aertrycke</i> serum	1:100	-	-	-	-	+	
	1:1000	-	-	-	-	+	
	1:2000					+	
	1:4000					+ p.	
<i>B. paratyphosus</i> β serum	1:100	+ p.	-	-	-		+
	1:1000	-	-	-	-		+
	1:4000	-	-	-	-		+

All reactions microscopic: time two hours. + p. = partial reaction.

The above table shows clearly that the four tested strains are identical with one another and are all identical with *B. enteritidis*.

The pathogenicity of the three strains R₁, R₃, R₄ was tested. All three were highly virulent to guinea pigs both on subcutaneous and intraperitoneal injection. Death in the latter case within 24 hours. Organisms recovered in pure culture from the spleen and other internal organs and completely identified with the injected bacilli.

In addition to the above bacilli culturally identical with *B. enteritidis* and other true Gaertner organisms a number of pseudo-Gaertner or para-Gaertner bacilli were met with. This name has been given by one of us to a group of organisms which culturally closely resemble true Gaertner group bacilli and which can only be culturally distinguished when an extended series of tests is employed, such as the fermentation of dulcitol, salicin and glycerine. They were not specially looked for in the present investigation and indeed by the use of the compound sugar test all the salicin pseudo-forms were cut out and could not be identified. The chief pseudo-Gaertner forms found were therefore of the dulcitol negative type, *i.e.* they were culturally identical with the true Gaertner strains except that they failed to ferment dulcitol. Such organisms were isolated from the intestinal contents of rats 7, 9, 12, 14, 18, 19, 26, 28 and 37. Perhaps of greater interest is the fact that these dulcitol negative strains were isolated in two cases from the internal organs, *i.e.* from the spleen of rat 19 and the heart blood of rat 25.

The chief interest attaching to these organisms is the fact that unless an extended series of cultural tests with these sugar-alcohol bodies is carried out they may easily be mistaken for true Gaertner organisms.

A further point of interest is the extent to which the sera of the rats react to Gaertner group organisms as evidence of old infection with

members of this group. Unfortunately we did not consider the importance of this point in our earlier cases and serum from only seven rats was collected.

These sera specimens were tested with all three varieties of the Gaertner group and also specially with Danysz's bacillus which is indistinguishable from and evidently identical with *B. enteritidis*. Tested in dilutions of 1:50 (time $1\frac{1}{2}$ hours, microscopic method) the sera of rats 36, 38 and 40 failed to agglutinate any of the four strains. The other four showed some agglutinative properties as follows.

Rat serum	Dilution	<i>B. suispestifer</i>	<i>B. para-typhosus</i> β	<i>B. enteritidis</i>	Danysz bacillus
Rat 27	1:50	+	+	+	+
"	1:100	+	+	+	+
"	1:500	+	+	+	+
"	1:1000	+p.	+	+	+
"	1:2000	-	-	+p.	+
"	1:5000	-	-	-	-
Rat 37	1:50	+	+	-	-
"	1:200	+p.	+	-	-
"	1:500	-	-	-	-
Rat 39	1:50	-	-	+	+
"	1:100	-	-	-	tr.
"	1:200	-	-	-	-
Rat 41	1:50	+	+	+	+
"	1:100	+	+	-	-
"	1:500	+	+	-	-
"	1:1000	-	-	-	-

All reactions microscopic: time $1\frac{1}{2}$ to 2 hours.

+p.=partial positive reaction. tr.=traces of reaction.

We do not think any significance can be attached to the slight reactions of the sera of rat 39 or possibly of rat 37 but the reactions obtained with rats 27 and 41 certainly point to an old infection with Gaertner group bacilli. Both these rats were obtained from the old refuse tips at Weston-super-Mare. From none of these rats were Gaertner group bacilli isolated.

CONCLUSIONS.

The results show that Gaertner group bacilli were only isolated from rats obtained in Weston-super-Mare. Inquiry showed that in November 1909, about $2\frac{1}{2}$ years before the first rats were examined, the refuse tips and slaughter houses had been extensively dosed with the Danysz virus. None had been used subsequently until May 1912.

On May 8th, 1912, twelve days before rats 12 and 13 were examined, twelve tubes of this virus were distributed in the slaughter houses while further tubes were used in other parts of the town. The Gaertner bacilli isolated from these rats which were found dying in the treated houses were obviously due to direct infection. The bacilli isolated from the other rats were all *B. enteritidis*, while Danysz virus is a true *B. enteritidis*.

It is difficult to resist the conclusion that these bacilli isolated from the rats were from an old—possibly years old—infection with this virus. If this be accepted they are evidence of an old infection and lend no support to the view that Gaertner group bacilli are natural inhabitants of the rat's alimentary canal. The completely negative results as regards finding these bacilli in the intestinal contents strongly support this view.

The persistence of bacilli in carrier cases has been noted by several observers. Thus Petrie and O'Brien (1910) succeeded in experimentally producing the carrier state in guinea pigs by feeding them with *B. suispestifer*. In the case of two animals (out of six fed) the bacilli were isolated from the faeces for as long as 59 days after the last feeding. In an epizootic amongst the stock guinea pigs at the Lister Institute due to *B. suispestifer* recorded by O'Brien (1910) the survivors showed definite immunity to this bacillus, and five of them proved to be carriers, excreting the bacillus intermittently five months later.

It is well known that rats fed with Danysz's bacillus often recover, and are then highly immune and can eat large amounts of virulent virus without apparent ill health. The ingested bacilli are present in large numbers in the excreta.

The few sera of rats tested are further evidence of old infection in certain cases.

Although no Gaertner group bacilli were isolated from the intestinal contents it is probable that for some time after infection these bacilli are excreted in the faeces. Rats frequently infest slaughter houses and there is considerable likelihood of the meat being infected with their excreta. That such excreta may contain actively virulent bacilli quite identical with *B. enteritidis*, the cause of so many outbreaks of food poisoning, cannot be considered satisfactory.

It is of interest to note that the Gaertner group bacilli isolated were highly virulent.

SUMMARY.

Forty-one rats examined—internal organs and intestinal contents—for presence of Gaertner group bacilli. Bacilli identical with *B. enteritidis* isolated from five rats in each case from the spleen (in two from liver also). No members of this group isolated from the intestinal contents.

Several of the rat sera were capable of agglutinating Gaertner group bacilli in high dilution. These facts point to the view that old infection with Gaertner group bacilli had taken place.

The general result of the investigation is in favour of the view that while rats are liable to be infected with Gaertner group bacilli and to be ill in consequence these bacilli are not natural intestinal inhabitants. If this be accepted it may be stated that this group of bacilli are not natural intestinal inhabitants, of any known animal species.

Rats infected with Gaertner group bacilli may serve as a means of infecting meat with these bacilli and may possibly in this way be a cause of meat poisoning outbreaks.

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THE EFFECT OF REPEATED BLEEDINGS ON THE BLOOD CONSTITUENTS OF IMMUNISED HORSES.

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(From the Wellcome Physiological Research Laboratories.)

(With 3 Charts.)

ONE of the phenomena in immunity not completely explained is the persistence of demonstrable antibody in patients for many years after recovery from an acute infection. A typical instance is that of a person maintaining a high agglutinin titre for years after an acute typhoid infection.

One obvious explanation that might suggest itself is that all such persons are "carriers" and that they are constantly being reimmunised by small doses of bacterial poison from the typhoid bacilli in gall bladder, intestine, or elsewhere. It is easy to extend such an hypothesis and to imagine that all immunity after natural infection is due to the persistence of the living agent of the infection in some concealed site in the body and the resultant frequent immunisation of the patient by the foreign protein of the infecting agent. But leaving such debatable ground and restricting the discussion to immunisation produced by non-living protein, we start with several facts.

A rabbit which has been passively immunised with rabbit or other serum containing antibody will lose practically the whole antibody from the blood in a few weeks or months (Jørgensen and Madsen, 1902).

This leads one to expect that *destruction or elimination* of antibody is constantly occurring also in the actively immunised animal. Since the titre in the latter case falls more slowly than does that of a passively immunised animal, one has to assume that antibody is constantly

appearing in the blood for a very long period after active immunisation to replace that destroyed, in other words, that antibody is being produced long after the probable disappearance of the antigen.

It has often been stated that antibody may be formed in response to the injection of non-specific antigen (Dreyer and Walker, 1909). An injection of dead staphylococci for instance is said to cause an increase in the titre of typhoid agglutinins in an animal immunised with *B. typhosus* and it is also stated that such non-specific influences as bleeding can cause the production of antibody (Roux and Vaillard, 1893). But as far as I can find, in all the experiments reported, *recently* immunised animals were dealt with so that circulating antigen was still possibly present and still able to exert its influence¹. Further, what was ascertained was the *titre* and not the total amount of antibody in the whole blood or in the whole of the tissues. Without these data it seems somewhat unsafe to conclude that antibody is actually produced anew.

Thus three procedures are really necessary before one can say that antibody has been produced as a result of bleeding, viz.

1. Estimation of titre,
2. " " total blood—and from these two factors, total
 antibody in the blood.
3. " " antibody in the tissues.

It may be advisable to refer briefly to certain experimental work on this question and to the conclusions reached.

*Summary of Previous Work on the Influence of Bleedings on
Antibody-content.*

Roux and Vaillard (1893) immunised two rabbits against tetanus toxin, ascertained the antitoxin-titre and then from one of them took 200 grms. of blood in the course of 20 days. They found that its titre two days after the bleeding had fallen only as much as that of the control immunised rabbit which had not been bled.

Salomonsen and Madsen (1898) immunised a mare against diphtheria and very shortly afterwards drew off seven litres of blood and found a drop in the antitoxic titre of the animal's blood and milk of about

¹ The theory that residual antigen gave rise to this apparently new production of antibody after bleeding was suggested by Rothberger (1906) though no definite experimental evidence was brought forward in its support.

one-seventh. They bled a second horse soon after injection, when the curve of antitoxin titre was mounting and noted a drop of 35%, but after a period of 12 days the titre was again at the previous level. In the course of seven days they took from a goat immunised with diphtheria toxin eleven-twelfths of its blood-volume and found a drop to 17% of the initial titre, but six days later it had reached 62% of the initial titre. Their conclusion was that the figures indicated a new production of antitoxin and that certain cells in the organism had acquired a new and persistent secretory power.

Nicolle (1904), working with rabbits, showed that the descent of the curve representing the titre of agglutinin for typhoid bacilli could be temporarily arrested by bleeding. Friedberger and Dorner (1905), in the case of rabbits immunised with goat corpuscles, bled the animals 10 to 20 c.c. either before or just after injection and found the titre in the bled animals was about four times that of the non-bled. In view of the therapeutic use of bleeding in the treatment of many diseases it is of interest to note that the favourable influence exerted by bleeding in these experiments was most marked where the bleeding was done two to three days after the injection.

Lüdke (1904) reported some results of bleeding in rabbits immunised with ox blood. He took very large amounts of blood, such as 80 to 100 c.c., from the rabbits at successive bleedings and apparently found that the titre became raised some days after the bleedings, but as he speaks of such quantities as "three to six drops of the serum" as being necessary to dissolve 1 c.c. of 4% ox blood corpuscles, one cannot draw very clear deductions from his work.

The same author (Lüdke, 1906) took 80 c.c. of blood in five days from a rabbit previously injected with ox blood, and one day later found the haemolytic titre unaltered. (Tables and full details were not given.)

Schroeder (1909), who reviewed the whole literature of the subject, described many experiments on rabbits immunised with *B. coli communis* and *B. typhosus*. He applied Madsen's equation for calculating any point on the curve of agglutinin development after a single injection. He then bled his rabbits and subsequently ascertained their titre and noted whether the point fell (*a*) on, (*b*) above, or (*c*) below the calculated point. His conclusion was that the bleeding had (*a*) left unaltered, (*b*) increased, (*c*) decreased the agglutinin content.

Bleeding was performed usually during the stage of falling curve and he found that the fall was delayed or even replaced by a temporary rise.

He therefore concluded that after such bleeding a considerable reproduction of agglutinin takes place. He apparently did no blood-volume determinations and did not investigate the distribution of agglutinin throughout the various tissues.

Dreyer and Walker (1910) stated that by repeated bleedings one can keep up the percentage of antibodies in the blood long after it would have fallen to a low level and can even cause an increase in the antibodies above the former maximum.

As a result of these various experiments by various workers clinicians have concluded that bleeding causes a rise in the agglutinin-titre and, making the natural assumption that a rise in agglutinin-titre is to the advantage of the patients, have taken from typhoid fever patients one or two hundred c.c. of blood in order to raise the agglutinin titre. This has been done by various physicians in Germany and by Schroeder in Denmark and has been recommended by Whitehead in England (1911).

Object of present experiments.

The experiments here brought forward deal with results observed after numerous bleedings. They were carried out on horses, with which animals estimation of total blood-volume or of the total amount of antibody in the tissues is not an easy matter, and also on rabbits, in which these estimations can be made.

Description of Experiments on the Horse.

Methods. 225 c.c. of a 7·5% suspension of sheep's red cells were injected into a horse which rapidly attained a high haemolytic titre. Two months later the haemolytic titre was found to be at a constant level over a period of three to four weeks. Various quantities of blood were then withdrawn at intervals of a week or more with occasional resting periods, so that in 11 months a total quantity of 122 litres was taken from the jugular vein.

Titration. After many trials with suspensions of red cells of varying strengths, readings after varying periods, etc., 0·5 c.c. of a 1% suspension of the centrifuged deposit of red cells, which deposit contained about 26 to 28 million cells per c.c., was mixed with 0·05 c.c. of fresh guinea-pig serum, 0·5 c.c. of the dilution of the horse's serum to be tested, and made up to 2·5 c.c. with 0·9% saline solution. The tubes were finally placed in a water-bath at 37° C. and the decisive reading was that taken

after one hour. It was not difficult to find the end point when each successive tube contained 20% less than the preceding one. When it contained 10% less, care had to be taken to have the surfaces of the tubes carefully freed from traces of dirt, but in bright daylight and with clean water in the bath it was generally possible to make a clear distinction between successive tubes.

The guinea-pig complement used was titrated each time with a fixed dose of red cells and of old stable haemolysin. The extreme limits of variation were between 0.01 c.c. and 0.014 c.c., but very rarely was the reading outside 0.01 c.c. to 0.02 c.c.

The individual specimens were tested as soon as possible after the bleeding and periodically batches of three, six or eight samples were titrated against each other. For the later bleedings an old stable haemolysin, obtained from another horse, was always used as a "standard," a procedure similar to the German method of estimating tetanus antitoxin.

In Table II the values of the haemolytic titre are recorded. Occasionally they will be seen to vary fairly widely, but where a wide discrepancy was observed numerous subsequent titrations were made of the same specimen, compared with previous bleedings and the "standard," and the average of the several readings is the point shown in Charts 1 and 2.

Red and white cells were enumerated with a Bürker haemocytometer, two duplicate samples of 1 c.c. of blood being usually counted. (Average error about 10%.) *Haemoglobin* was estimated in duplicate or triplicate with a Gowers instrument—human scale. (Average error about 3%.) For *specific gravity* two bottles, each containing 10 c.c. of blood, were weighed. The blood was caught from a cannula in the vein and immediately filled into the bottles. These were kept at laboratory temperature, which varied from about 10° C. to 20° C. From Table II it will be seen that the extreme difference between the duplicate readings was two in the fourth figure. Taking the last two figures of readings of the samples to represent comparative percentages this gives an average error of about 4%. For *differential counts* usually about 400 white cells were counted, checked at important points by two such counts. (Average error about 10%.)

The total proteins were estimated by refractometer readings checked and interpreted by coagulating the total proteins and weighing the precipitate at many points. (Average error about 2%.)

TABLE II.

Effect of repeated bleedings on constituents of blood of immunised horse.

HORSE 1.												
Date	Amount bled litres	Red cells per c. mm.	Haemo- globin	Specific gravity	Total protein	Leuco- cytes	Differential count					Haemo- lytic titre
							total counted	polymorpho- nuclear cells (%)	mono- nuclear cells (%)	eosinophile (%)	mast cells (%)	
6. 2. 12	4	10,200,000	95.1	1048	6.45	9100	169	59	35	7		.0016
		100	100	100	100	100						
10. 2. 12		9,600,000	83			9400						.0016
		94	87			8550						
						100						
15. 2. 12		9,040,000		1047	6.45	8650						
		89		97	100	8800						
20. 2. 12	4			1047	6.5	98						
				97	100.5							
22. 2. 12		10,200,000		1046	6.31	11400						.0016
		100		95	98	10200						
						118						
1. 3. 12	4	8,650,000	80	1046	6.32	7250	472	60	34	6		
		8,300,000	84	95	98	81						
		84										
8. 3. 12	4	8,160,000		1046	5.81	8150						.0030 (?)
		80		95	90	91						.0016
												.0016
15. 3. 12		7,680,000	83	1049	6.39	10450	576	69	26	4	1	.0016
		75	87	103	99	117						
20. 3. 12	9	9,660,000			6.41	7200						.0016
		94			99.5	79						
22. 3. 12		8,460,000		1046		10000	314	70	27	3	1	.0016
		8,620,000		95		10350						.002
		84				113						
25. 3. 12							308	65	30	5		.0008 (?)
												.0016
												.0016
2. 4. 12		9,780,000	90	1055	6.91	7600	441	68	26	5		.001
		95	95	1054	112	86						
				113								
17. 5. 12	4	10,400,000		1059		11000	437	68	23	8	1	.0016
		9,930,000		1059		124						
		100		122								
23. 5. 12	4	9,920,000		1052		7900						.0016
		97		1052		89						
				108								

Haemolytic titre = the amount in c.c. of serum necessary to completely dissolve .5 c.c. of 5% red cell suspension with .05 c.c. fresh guinea-pig serum in one hour at 37° C.

The figures in heavy type represent percentages.

Date	Amount bled litres	Red cells per c.mm.	Haemo-globin	Specific gravity	Total protein	Leuco-cytes	Differential count					Haemo-lytic titre
							total counted	polymorpho-nuclear cells (%)	mono-nuclear cells (%)	eosinophile (%)	unst. cells (%)	
29. 5. 12	4	9,408,000 8,605,000 88		1041 1041 85		7200	392	76	19	4		·0016
3. 6. 12	4	8,000,000 79		1046 95		7300	378	71	26	3	1	·0016
11. 6. 12	6	9,970,000 97	84 86	1044 1045 91		13200						
17. 6. 12	6	8,320,000 8,080,000 81	70 73	1048 1048 91		6400 6900 74	446	67	31	2		
24. 6. 12	6	7,500,000 7,280,000 73		1044 1045 1044 91	6·21 96·5	8000 8600 83	442	68	28	3	1	·0016 ·0016
2. 7. 12	6	7,080,000 6,640,000 67	84 88	1046 1046 1044 92	6·35 98	6660 6700 74	423	71	25	3	1	·002 ·0016
2. 9. 12	5	9,520,000 8,880,000 89	90 95	1052 1054 110		4400 5300 56						·002
10. 9. 12	6											·002 ·0016 ·0024
27. 9. 12	6	7,960,000 8,320,000 80	76 80	1043 1045 92	6·35 98	5600 4800 60	350	69	23	7	1	·002 ·002
16. 10. 12	6				6·61 102							
25. 10. 12	6				6·48 100·5							
2. 11. 12	6				6·21 96							·0024
8. 11. 12	6				6·11 94·5							
16. 11. 12	6	6,770,000 8,000,000 72	75 79	1044 91	6·2 96	6660 6400 73	430	70	22	7	1	·0024
19. 12. 12	10	8,800,000 87	104 109	1057 118	7·2 111	6000 68						·0024
31. 12. 12		6,320,000 6,160,000 61	74 73	1047 97	6·7 104	8600 8500 92						
3. 2. 13		8,160,000 80	93 95	1053 110	6·8 105	6000 68						

experiment. *Haemolytic titre* was but slightly affected¹. During the year (1912) the titre has gradually dropped to 66% of the original, the fall commencing in June, by which time 40 litres had been withdrawn, and being accelerated by the September, October and November bleedings which amounted to 58 litres.

Other constituents. The red cells and haemoglobin (the colour index varying very little from unity) and the specific gravity showed considerable fluctuations and at the end of the experiment are at a somewhat lower level than at the commencement. The power of maintaining the protein content is very striking and the horse at the end of 12 months has a higher protein content than at the beginning of the bleedings.

The rapid rise in all constituents during the resting periods April-May and July-August shows how active the power of repair remained. This ability to undergo repeated large bleedings of eight litres each with but slight subsequent fall in blood constituents is also seen in Table III and Chart 3 taken from another horse under observation (horse 2).

TABLE III.

General results of repeated bleedings amounting to 197 litres = four to five times total blood-volume, in a period of 11 months.

HORSE 2.

	Specific gravity	Red cells per c.c.	White cells per c.c.	total counted	Differential count				Haemoglobin	Total protein (%)
					poly-morpho-nuclear cells (%)	mono-nuclear cells (%)	eosino-philic (%)	mast cells (%)		
25. 3. 12	1055	9,000,000	8800	334	60	34	6	0	74	8.0
Total bleedings 64 litres	100	100	100						100	100
3. 7. 12	1039	6,288,000	8800	422	71	24	4	0	70	7.2
Total bleedings 48 litres	71	70	100						95	90
27. 9. 12	1044	6,840,000	6,530						70	7.5
Total bleedings 36 litres	80	76	85						95	93
18. 11. 12	1042	6,800,000	10000	511	59	35	5	1	64	6.3
Total bleedings 49 litres	76	76	115						86	79
18. 2. 13	1045	6,400,000	8000						65.6	6.8
	82	70	91						88	65

The figures in heavy type represent percentages.

¹ From results published elsewhere (*Journ. of Path. and Bact.* xviii. 90) it appears that this horse can keep its haemolytic titre curiously constant even after a 10 litre bleeding.

The relation between Antibody Curve and that of other constituents of the blood.

To revert to a consideration of the results obtained with horse 1 it was hoped that a study of the curves would reveal a close association between one or other of the various blood constituents and the antibody and so perhaps suggest a site for the manufacture of antibody—but no such association appears. The only constituent that departs widely from the haemolysin curve and that of the other constituents is the white cell content, which fact is of interest, when one recalls the view so often advanced that the leucocytes or the tissues where they originate are the main sites of antibody manufacture.

The one interesting fact that emerges is that although in a series of bleedings commenced three months after the last injection of sheep's red cells, 122 litres of blood were taken from the horse its haemolytic

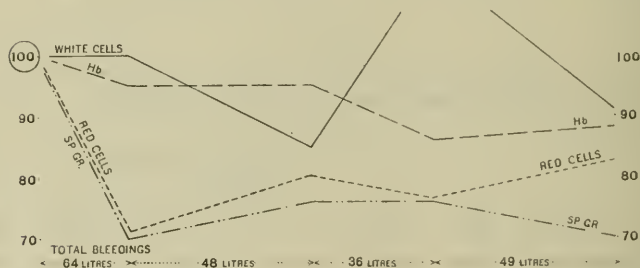


Chart 3. Effect of repeated bleedings on blood of horse 2. Constituents expressed as percentages of figures at start of experiment. A total of 197 litres withdrawn in 11 months.

titre is still 66% of the constant titre attained before the bleedings were commenced. This amount of blood probably represents $2\frac{1}{2}$ to $3\frac{1}{2}$ times the total blood-volume. This assumption is founded on the following experimental data:

(A) In a series of some 30 horses bled out from the carotid artery the average amount of blood obtained was 27 litres. From a rabbit or dog one can bleed out $\frac{3}{4}$ of its total blood-volume. The use of this factor of $\frac{3}{4}$ would make the total blood-volume of the average horse about 36 litres.

(B) One can inject 5 to 10 litres of saline into one jugular vein of a horse in about 8 to 14 minutes, and immediately afterwards take a sample from the opposite jugular and estimate the fall in the content of red cells, haemoglobin, total proteins or specific gravity. By treating the experiment as a dilution *in vitro* and by making no allowance for the unknown rate at which absorption and elimination immediately proceed, the blood-volume may be calculated. This procedure has given me figures like 42, 45, 51 and 53 litres. I am endeavouring at present by Schürer's modification of this injection method to obtain better figures than these, but meanwhile assume that the average horse contains 35 to 45 litres of blood.

It is certain that the initial figure would have dropped somewhat during the year, apart from any interference, so that we can safely conclude

(1) That the series of bleedings has had only a limited adverse influence on the titre.

(2) That the horse has either produced afresh something like three times the amount of antibody in the blood stream at the commencement of the bleedings or that this amount of antibody may have come from some pre-existing store of antibody in the tissues.

The Question of a Reservoir of ready made antibody in the tissues or lymph.

This question was investigated on rabbits immunised long previously with sheep's red cells and therefore possessing a fairly constant titre. Four experiments were performed. In one (*v.* Table IV) the rabbit had not been previously bled more than a few c.c. at a time; the other three had been subjected to one or two bleedings of about 20 c.c. each, during the week preceding the experiment. Each of these preliminary bleedings was followed by such a production or a readjustment of distribution of antibody that the haemolytic titre remained practically constant and it was hoped that if the faculty of storing in the tissues ready made haemolysin existed, such faculty would be enhanced in response to the bleeding and so render the storehouse more easily detected.

Technique. The rabbit was bled out from the carotid and then about $2\frac{1}{2}$ litres of Ringer's fluid were run through from the aorta. The washings, obtained from the right auricle, were caught in successive jars in each of which the red cells and antibody were estimated; the spleen, liver, appendix, kidneys, brain and samples of muscle and marrow

were ground up separately and extracted with saline solution. Each extract was tested for antibody.

The results are given in Tables IV, V, VI and VII. Making allowance for the difficulty of estimating haemolysin accurately in these washings, it seems fair to conclude that there is no great store of ready made detectable haemolysin that can be washed out or extracted by grinding and washing¹.

It was conceivable that haemolysin was present in these organ extracts but not detected because of some inhibitory principle also

TABLE IV.

*Haemolytic rabbit bled out, then washed out with
2 litres of Ringer's fluid.*

	Diluted blood c.c.	Percentage of red corpuscles	Volume of blood c.c.	Percentage of total blood	Percentage of total haemolysin
Jar 1	—	100	45	60	75
Ringer commenced					
Jar 2	250	7.2	18	24	13
„ 3	750	1.6	21	14	8
„ 4	250	.51	1	1	2
„ 5	500	.1			1
		organs and limbs massaged			
„ 6	250	.2			.3
„ 7	100	.3			.05

TABLE V.

*Haemolytic rabbit (bled 40 c.c. during previous week) bled out, washed
out with 2½ litres of Ringer's fluid, organs ground up and
haemolysin content determined.*

	Diluted blood c.c.	Percentage of red corpuscles	Volume of blood c.c.	Percentage of total blood	Percentage of total haemolysin
Jar 1	—	100	65	72	44
„ 2	150	7.5	10	11	10
„ 3	540	1.86	10	11	9
„ 4	1280	.32	4	4	21
„ 5	540	.25	1	1	12

Muscle, appendix, brain, spleen, liver, kidneys together contained about 4

¹ Dreyer and Ray (*Journ. Path.* xiii, p. 344, 1909) state that they were able to wash out of the tissues 30 % of the total agglutinins obtained. These results apparently do not run parallel with those obtained from rabbits long previously immunised with red cells.

TABLE VI.

Rabbit (bled 40 c.c. during previous week) bled out, washed out with $2\frac{1}{2}$ litres of Ringer's fluid, organs ground up and haemolysin content determined.

	Diluted blood c.c.	Percentage of red corpuscles	Volume of blood c.c.	Percentage of total blood	Percentage of total haemolysin
Jar 1	—	100	54	79	98.2
„ 2	260	1.4	3	4	1
„ 3	1000	.82	8	12	.6
„ 4	1300	.26	3	4	.1

Appendix, brain, spleen, liver, kidney and muscle together contained about 1

TABLE VII.

Rabbit (bled 25 c.c. two days previously) bled out, washed out with 4 litres of Ringer's fluid, organs ground up and haemolysin content determined.

	Diluted blood c.c.	Percentage of red corpuscles	Volume of blood c.c.	Percentage of total blood	Percentage of total haemolysin
Jar 1	—	100	70	76	61
„ 2	500	2	10	11	8.6
„ 3	500	1.1	5	5	6.5
„ 4	1000	.3	3	3	10.6
„ 5	500	.1	.5	.5	.8
„ 6	1425	.3	4	4	12.4

Liver, spleen, kidney, muscles and marrow together contained about .8

present in the extract. To test this a normal rabbit's tissues were ground up and a certain volume of the paste of various organs mixed with an equal volume of rabbit serum containing a known quantity of anti-sheep haemolysin and allowed to remain in contact for some hours. The haemolysin in the supernatant fluid was again estimated, but no evidence was found of any marked inhibitory influence in pastes of spleen or muscle and only slight inhibition was caused by liver paste.

Summary of Results.

(1) From a horse injected with sheep's red cells three months prior to the first bleeding and in a condition of constant haemolytic titre 122 litres of blood were taken in a period of 11 months. The horse's condition remained good throughout and has markedly improved during the year.

(2) The net result was that the haemolytic titre during that time fell only to about 66% of its original value, the leucocytes to about 66%, haemoglobin scarcely at all, while the specific gravity of the blood and total protein have increased, the former by 10%, the latter by 5%.

(3) There was no relationship between the total number of leucocytes¹ and the amount of antibody. The differential count showed an increase of 12% in the polymorphonuclear and a decrease of 12% in the mononuclear cells, these figures being not very far outside the experimental error. The eosinophile and mast cells showed no marked alteration in number, size or staining reactions.

Discussion of Results—Conclusions.

(1) A horse can be trained to withstand without any recognisable ill effect on its general health, and with remarkably little diminution in blood constituents, very large bleedings, amounting to several times its total blood-volume in less than a year. By allowing a considerable time to elapse after injection, it was expected that one would get further away from the effect of the presence of antigen and so hope to obtain a clear view of the effect of bleedings.

Whether a foreign protein may resist destruction in the body for months we do not know. But it is not probable, for if one injects by any parenteral method a serum containing an antibody, *e.g.* diphtheria antitoxin, it is found that in a very short period such antibody is no longer detectable and has presumably been destroyed. We know further that when one injects an easily recognisable foreign protein such as nucleated bird corpuscles into a mammal, the nucleated foreign cells rapidly disappear from the blood stream and undergo phagocytosis in the haemopoietic tissues.

It is therefore probable that the sheep cells injected in October 1911 had been eliminated or destroyed before February 1st, 1912, and that any subsequent alterations of haemolytic titre were not dependent on residual antigen. Experiments on rabbits (*v.* Table I) showed, firstly, that antibody (haemolysin) may remain nearly constant in titre after bleeding though the blood-volume (Boycott, 1909) increases, and, secondly (*v.* Tables IV, V, VI, VII), that the amount of ready made haemolysin in the tissues is negligible.

¹ It was not possible to take samples at the same hour every day. They were taken between 10 a.m. and 5 p.m. but mostly about 3 p.m.

TABLE I.

Small alterations of haemolytic titre following bleeding rabbit 1, bled 20 c.c. on Sept. 9, 1912.

Technique. Guinea-pig serum 0.05 c.c., 1 % red cell emulsion 0.5 c.c., saline added to 2.5 c.c., water-bath at 37°, reading after one hour.

Quantity of serum	Sept. 9, 1912	Sept. 10	Sept. 11	Sept. 12	Sept. 13	Sept. 14
0.0016 c.c.	##	##	##	##	##	##
0.00128	##	##	##	##	##	##
0.00102	#	#	##	#	#	#
0.0008	#	#	#	+	+	+
0.0006	+	+	+	±	±	+

= complete haemolysis.

± indicate grades of haemolysis.

+ = very slight haemolysis.

This table is typical of five such experiments, the amounts bled being from 15 c.c. to 35 c.c.

We may assume then that there was no storehouse or reservoir of formed haemolysin in the tissues on which the horse could have drawn during the eleven months of the experiment, and since $2\frac{1}{2}$ to $3\frac{1}{2}$ times its total blood-volume was withdrawn, one may consider the conclusion definitely proved that

(2) The cells of an immunised animal have acquired a persistent faculty of manufacturing haemolysin and can do so in response to (or possibly in spite of) repeated extensive bleedings.

With regard to the site of production of antibody, it is difficult to correlate the haemolysin production with variations in the quantitative proportions of cells of the haemopoietic system or of the protein constituents of the serum.

I have to express my gratitude to my colleagues Dr Laidlaw and Dr Walpole, to the former for instruction and assistance in the washing out of the rabbits and to the latter for performing the whole of the protein determinations.

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THE BLOOD VOLUME IN ANKYLOSTOMIASIS.

WITH SOME BIOLOGICAL NOTES RELATING TO THE DISEASE.

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Introductory.

THE investigations which form the subject of the present paper were carried out during my tenure of the Ernest Hart Memorial Scholarship in State Medicine of the British Medical Association. The experimental part of the work was conducted at the Lister Institute of Preventive Medicine, London.

The suggestion of the line of research came from Dr J. S. Haldane, F.R.S., who referred me to certain results obtained by Boycott and himself (1903). The essential question to be decided was the nature of the changes in the blood volume in Ankylostomiasis or hook-worm anaemia. In the paper referred to Boycott and Haldane, using the carbon monoxide method of estimation, found that in three patients suffering from hook-worm anaemia the blood volume was increased to a very considerable extent, while the total haemoglobin remained practically unchanged. The important bearing of this result upon the nature of anaemia in general appeared to demand confirmatory evidence and it was thought that such might be readily obtained by means of animal experimentation.

In setting out to deal with such a problem experimentally, several questions naturally suggested themselves. These were, in the first place, the exact nature of the disease as it is exhibited in man, its etiology, pathology and clinical identity; in the second place, the nature of the disease in the animals to be experimented upon and the question as to whether it is identical with, analogous to, or different

from the disease in man. In addition there was the question as to the possibility of accurately determining normal conditions in the experimental animals and finally various questions relating to idiosyncrasy, susceptibility and immunity.

With regard to the nature of the disease in man it is universally accepted that the prime etiological factor is the presence in the intestine of either or both of two species of parasitic worms, namely *Agchylostoma¹ duodenale* and *Necator americanus*. As to the direct or immediate cause of the anaemia, however, opinion is yet to a large extent empirical. From the habits of the worms of fixing on to and damaging the intestinal mucosa, and so leading to haemorrhage, it might at first sight be concluded that the causation of the anaemia could be fully explained by the resulting loss of blood. The anaemia would accordingly be of the secondary or haemorrhagic type. Against this, however, must be put certain facts of considerable weight. In the first place Looss (1911) insists that the worms do not suck blood and that their natural food is the intestinal epithelium. This view, in the latter respect, is supported by several observers, and by personal experience, to the effect that although blood is not infrequently found in the intestine of the worms the red blood corpuscles are not digested to any extent. In the second place the majority of observations point to the fact that intestinal haemorrhage is rare in cases of some duration, and thirdly the results of Boycott and Haldane give evidence that the blood content differs from that in secondary anaemias. These considerations, together with some of a more general nature, have led to the origin of the toxic theory. This in turn has of late developed along two distinct lines. On the one hand, it was believed that the worm itself secreted a toxic substance, the action of which gives rise to the anaemia; on the other hand, there is the more recent idea that the toxin is produced by some extraneous organisms such as intestinal bacteria, the products from which find their way through the damaged mucosa. Neither of these explanations, however, is entirely satisfactory. A number of attempts have been made to isolate the toxic substance and success has been reported by, amongst others, Preti (1908), Noc (1908) and Whipple (1909). The last mentioned, however, while confirming the presence of a haemolytic substance, maintains that it is too weak or present in too small quantities to be effective. Moreover the evidence that haemolysis does actually take place within the human body is conflicting and the latest observations by Ryffel are against the occurrence of any such

¹ This spelling is adopted in accordance with current zoological nomenclature.

process. Loeb and Smith (1904 and 1906), again, entirely failed to detect the presence of a haemolysin in the head of the worm, but at the same time they reported the presence of an anti-coagulating substance, which might possibly tend to favour the continuance of haemorrhage. The presence of this substance was confirmed by Noc (1908), but Liefmann (1905) could detect its presence only in a minority of cases.

The toxic theory of extraneous origin has of late been elaborated by Weinberg and Leger (1908). Their opinion is that the gravest forms of hook-worm anaemia can only be explained on the basis of a secondary microbic infection and they advance experimental proof in support of their belief. Whipple (1909), also, in discarding the haemolytic theory, comes to the conclusion that the anaemia is probably due to direct loss of blood accompanied by absorption from secondary foci of inflammation in the intestinal wall. Siccardi (1910) in a summarizing article concludes that the anaemia is chiefly of toxic origin but that the intestinal lesions and the haemorrhages are additional factors. Evidence from a different point of view is brought forward by Castellani (1910), who deals with the comparative frequency of fever in hook-worm infection in Ceylon. This fever he ascribes to the action of various intestinal, but not necessarily pathogenic, bacteria, amongst which a new form, *Bacillus asiaticus*, is particularly mentioned. The greatest objection, perhaps, to be raised against this secondary microbic theory is the rapidity with which the anaemia disappears on expulsion of the worms.

In dealing with the clinical aspects of the disease, one important point has to be borne in mind, namely, the distinction between hook-worm infection and hook-worm disease. Infection with the hook-worm, even in some cases to a very high degree, does not necessarily lead to disease. This fact has been observed by most workers who have studied the disease on a large scale and it is merely an instance of a general biological phenomenon, which is not capable of very ready explanation. Clinically the leading feature of hook-worm anaemia in man is the chronic and progressive nature of the disease, but although essentially chronic, the progress of the disease may in some cases be very rapid. The symptoms are those of severe anaemia, generally associated with some degree of gastro-enteritis. The large number of individual symptoms which may occur are not specifically characteristic of hook-worm anaemia. In the pallor and oedema, however, there is a greater resemblance to chlorosis than to pernicious anaemia. The blood shows

a more characteristic picture. The volume, according to Boycott and Haldane, may be increased by nearly 100 %, while the total oxygen capacity is only slightly decreased. The number of erythrocytes may fall as low as 1,000,000 per c. mm. while the haemoglobin percentage may be as small as 15 % or even 10 %. The colour index is generally considerably below unity. It is apparent that these facts give evidence of a close relation to secondary anaemia, the one essential difference being the fact that the total oxygen capacity is not materially diminished.

With regard to the blood cells several observers have remarked on the frequent occurrence of normoblasts and, in some cases, of megablasts. Boycott (1911), however, categorically denies the occurrence of such cells and argues, from their absence, that there is little regeneration of blood, "presumably because the blood contains as much haemoglobin as, and more red cells than, normal." In regard to the leucocytes the most important feature is the occurrence of eosinophilia. This was first noted in hook-worm anaemia (1891) by Mueller and Rieder, but since then it has been a matter of frequent remark, and it is now fairly well established that a high degree of eosinophilia frequently accompanies not only hook-worm anaemia but also hook-worm infection. At the same time it must be noted that the relation is not constant and that certain cases may show no eosinophilia even though heavily infected. This was pointed out by Low in the discussion on Boycott's paper in 1905 in the particular case of the natives of Uganda. It was found by Boycott (1911) that eosinophilia first became marked about two to three weeks after the initial infection and that it reached a higher degree about the time ova first appeared in the faeces, after which it tends to fall off but usually persists not only throughout the whole course of disease but even for many years after complete recovery.

Apart from bronchial asthma and scarlet fever, there are two classes of infection with which eosinophilia is particularly associated, namely parasitic worms and skin diseases. There is at present no satisfactory explanation of this remarkable association but the current theory is that the increase in the eosinophil cells betokens a reaction on the part of the marrow to toxins circulating in the blood. Such a theory is supported in the case of parasitic worms by the fact that encysted forms are frequently surrounded by a zone of cells, a large proportion of which are eosinophilic. Whatever the explanation, however, there can be no doubt that the occurrence of eosinophilia is not infrequently a useful

aid to diagnosis and it has indeed been employed by Boycott as a routine method in the case of miner's anaemia, the final and confirmatory diagnosis being made on examination of the faeces.

The disease in dogs and other animals.

With these facts in the case of the disease in man it is necessary to compare what is known in regard to similar conditions affecting animals, and here, as might be expected, the available facts are neither so numerous nor so carefully sifted. It is in the first place necessary to consider the class of worms to which the human hook-worms belong, for it has frequently happened that much confusion has been introduced from neglect of accurately determining the specific characters of the infective agent. In this wise epidemiological misconceptions have from time to time been promulgated, such, for instance, as the belief that dogs and other animals serve as carriers of the human hook-worms, or that the hook-worm passes through a sexual stage outside the body. *Agchylostoma duodenale* and *Necator americanus* may be characterised as bursate or Strongylid Nematodes, belonging to the family Agchylostomidae, the chief features of which, separating them from other bursate Nematodes, are the dorsal bending of the head, the presence of a large buccal capsule which is armed with symmetrical groups of teeth or cutting plates, but which lacks coronae radiatae. These characters are sufficient to differentiate the hook-worms from the Sclerostomes, on the one hand, and, on the other, from the "wire-worms" and lung worms of sheep and other Herbivora. It may be remarked that a considerable proportion of all those worms are capable of provoking haemorrhage and giving rise to anaemia and malnutrition. The Agchylostomidae, however, are regarded as particularly deserving of the title "blood suckers," although, as has already been pointed out, the term is possibly a misnomer. They are divided into two subgroups, *Agchylostominae* and *Bunostominae*, the former being parasites of Carnivora, the latter of Herbivora. The chief structural differences between these groups consist in the facts that the *Bunostominae* have an extra pair of internal teeth and an unpaired internal dorsal prominence (the so-called unpaired dorsal tooth of *Necator*) in the buccal capsule. *Agchylostoma* belongs to the first group, *Necator* to the second. As mentioned above it has from time to time been stated that *A. duodenale* occurs in the dog and other animals but it is now fairly conclusively established that this species is a specific parasite of man. The dog, the cat and the fox,

however, and probably some other Carnivores harbour a very similar species, *A. caninum*. Four additional species have been recorded from other Carnivores. Another genus, *Uncinaria*, contains a second parasite of the dog and cat, namely, *U. criniformis* and one or two other species occur in other Carnivores. *Necator americanus* is nearly as specific as *A. duodenale*, but it has been found, with certainty, in the gorilla. A second species, *N. africanus*, has been met with in the chimpanzee. The second genus of this group, namely *Bunostomum*, includes three species parasitic in cattle and sheep. Five other genera, comprising forms parasitic in various Herbivora, belong to this group. These include all the forms on which we have at present satisfactory information, but there are several others still imperfectly known.

That most of these parasites give rise to some form of anaemia is vouched for by several observers. *Bunostomum phlebotomum*, for instance, is credited (Ransom 1911) with being the cause of "salt-sickness" of cattle in Florida, which is characterised by, amongst a variety of other symptoms, progressive emaciation and pronounced anaemia, which in many cases terminates fatally. Like opinions in regard to the other species are not wanting, but it is chiefly with the disease as manifested in dogs that we are at present concerned. A form of pernicious anaemia in dogs, more particularly hunting dogs, has been known for a long time and its connection with hook-worm infection was first mooted in 1882 by Mégnin whose opinion was supported by Railliet and by Trasbot (1882), and was confirmed by further observations on the part of Mégnin (1883). From these we gather that the symptoms are as a rule much more severe than those in human hook-worm anaemia, and that the disease is on the whole less chronic and more fulminant in type. There is a similar debility, pallor, oedema and enteritis but there appears to be a greater tendency to vicarious haemorrhage especially in the form of epistaxis, and, to this, skin affections are added. That all the symptoms mentioned by these early authors constitute a single clinical entity must be a matter of some doubt. It is recognised that dogs are subject to anaemia of non-parasitic origin and it may be noted that in some of the cases of hook-worm anaemia only a few worms were found in the intestine. It is remarkable that the disease should only attack kennelled hunting dogs although it cannot be denied that the circumstances are especially favourable to continued and repeated infection. Later observations on this subject are scanty. Gray (1899) recorded the occurrence of ankylostomiasis in dogs in Assam and asserted that very profuse

haemorrhage was frequent. Powell recorded somewhat similar facts for Cochin China. Thiroux and Teppaz (1906) also record the occurrence of hook-worm disease in dogs in West Africa. It is not made clear, however, what proportion of the infected dogs the disease attacks or what the age-incidence or other epidemiological factors are. According to the observations and experimental work of Looss (1911) it would appear that only young dogs are liable to attack and Liefmann (1905) noted experimentally that older dogs resisted infection to a very considerable extent. A similar anaemic disease in cats was first observed in 1878 by Grassi.

With regard to the condition of the blood in the anaemia of dogs we have practically little or no information beyond the fact that the erythrocytes are diminished and the leucocytes and eosinophils increased.

From the foregoing remarks it is evident that idiosyncrasy and susceptibility play a certain, if little understood, part in the etiology of hook-worm infection and anaemia not only in man but also in dogs and probably other animals. In regard to hook-worm infection in man, age appears to have little importance, but in many animals and with other parasites besides hook-worms, only young animals are prone to infection. The same phenomenon is manifested in the case of the human thread-worm (*Oxyuris vermicularis*) which shows an overwhelming predilection for children and generally disappears after puberty. In the case of the human hook-worm however, idiosyncrasy is manifested in other directions in illustration of which may be quoted the fact that Boycott was apparently insusceptible to infection through the skin although he readily acquired infection through the mouth.

The blood-picture in normal dogs.

There is finally the consideration of the normal condition in the experimental animals. The blood-picture of dogs and cats has been dealt with by Paton, Gulland and Fowler (1902). They found that, normally, the dog has a wide range in the number of red blood cells, namely, from 5 to 9 millions per c.mm., the leucocytes from 11,000 to 26,000, and the haemoglobin index from 80 to 110. They also found an average of 3 to 4% of eosinophils. My own observations give figures agreeing in the main with these, namely, reds 5-8 millions, leucocytes 6-24 thousand, and Hb percentage 70-105, while the eosinophils vary from 0 to 4%. With regard to the normal blood volume several authorities have made observations which show a fair

amount of uniformity but diverge in respect of the limits. Ranke arrived at an average of $1/15$ of the body-weight, Jolyet and Laffont gave as limits $1/12$ to $1/13$; Pannum found $1/12$ – $1/15$ and Heidenhain $1/12$ – $1/18$. These latter figures are quoted from Dreyer and Ray (1910), the original references not being available. More recently Abderhalden and Schmid (1910) have employed an optical method of estimation, in which they injected dextrin and determined the rotatory power of the blood before and after injection. By this means they found the blood volume to be $1/8$ – $1/9$ (11.3 – 12.4%) of the body weight. Dreyer and Ray, however, maintain that the blood volume is a function not of the body weight but of the body surface (*i.e.* of the $2/3$ rd power of the body weight approximately). They express their results in the form $B.N. = B.W. \frac{2}{3}/k$, where k is a constant determined experimentally for each species. It is evident that considerable discrepancy exists between the results of Abderhalden and Schmid and those of other observers. My own determinations on normal dogs give evidence of a comparatively wide range, the figures being $1/16$ – $1/10$ (6 – 10%) of the body weight, and this apparently independent of age or size. It has been found by Boycott (1912) and also by Dreyer and Ray (1910) that the blood volume per kilo of body weight is on the whole highest in small, *i.e.* young animals, and that it tends to decrease, though not periodically, as the animal increases in size. Boycott also concludes that the total haemoglobin (*i.e.* total oxygen capacity) is highest in small animals, although the haemoglobin percentage tends to rise as the animal grows.

Source of culture employed.

In view of what has been stated above and as a matter of expediency it was decided to conduct the experimental part of this investigation upon dogs, and, if possible, cats. Hook-worm disease in dogs is not known to occur naturally in this country and on that account some initial difficulty was experienced in obtaining the material for infection. This I eventually owed to the kindness of Prof. C. W. Stiles of Washington, U.S.A. A sample of egg-laden faeces from an infected dog was sent, and numerous eggs survived the journey of nearly a fortnight. From these a successful culture of infective larvae was obtained.

Method of estimation of blood volume.

Before proceeding to the determination of the blood volume in the infected dogs, preliminary control estimations were performed on three normal cats and five normal dogs. The method employed was a modified Welcker process, the details of which are as follows. The animal was carefully weighed, narcotised with morphine and then chloroformed. The carotid artery on one side was exposed and a three-way cannula was inserted. To this was attached a length of rubber tubing leading from the bottle containing the washing out fluid, and another clamped rubber tube by which the blood could be drawn off. The washing out fluid consisted of a 0.4% solution of potassium oxalate in 0.75% solution of sodium chloride in distilled water, which was kept at a temperature of 37° C. An accurately measured quantity of blood was then drawn off (25 c.c.), and mixed with an equal quantity of oxalate solution. This served as a standard. A further quantity of blood was then drawn off into a vessel containing oxalate solution, and this was continued for 5–10 minutes or until the flow became slow. Oxalate solution was then driven into the circulation from a pressure bottle, which was placed about six feet above the operating table. The solution was allowed to flow for 5–10 minutes, when more blood was drawn off. This was repeated several times for about an hour, by which time the heart had ceased to beat and the fluid drawn off had become somewhat pale in colour. The carotid artery was then clamped and the chest opened. A cannula was inserted into the aorta and connected with the oxalate bottle; the right auricle was incised. Oxalate solution was then perfused through the circulation and the fluid returning from the right auricle was collected in the thoracic cavity, whence it was sucked by means of a water pump into a collecting bottle. All the while the muscles and liver were firmly massaged. The lungs were completely washed out by a similar procedure. This process was generally discontinued after about two hours, by which time the fluid washed out, although it had not entirely lost colour, had become extremely pale. All the washings were then mixed and the total accurately measured. About a litre of this was retained for estimation. Meanwhile the animal was skinned and the flesh removed from the bones, along with the liver, spleen, heart and kidneys. The flesh and the bones were mashed up separately and then pressed to expel the juice which was collected and measured.

The standard sample of blood which had first been drawn off was then diluted to 75% with distilled water to which a few drops of chloroform were added to ensure haemolysis. The washed out blood was also diluted and haemolysed, and a small quantity was passed through filter paper to remove suspended matter. By this means a clear solution was obtained without losing more than a trace of the colouring matter. The difficulty of obtaining a clear solution from the pressed muscle juice was met by saturating it with sodium chloride and filtering. Allowance was made in the calculations for the increase in volume resulting from the salt added.

Each of these solutions was compared with the standard. Three test tubes of equal calibre were employed for the estimation. Into two was placed a quantity of the standard sample and into the third a measured quantity, usually 10 c.c., of the diluted washings. To this was added distilled water from a graduated burette until the tint matched that of the standard. The amount of water added was noted and from this the strength of the solution was estimated. The total volume could then be calculated.

In some of the earlier estimations the washings were collected in two or three successive portions and each was estimated separately. It was found that the last portion contained so little haemoglobin that no material addition would have been made by continuing the washing process longer. In the case of the muscle juice the presence of muscle pigment rendered the solution brown so that it was impossible to compare it accurately with the standard solution. On that account the estimation was made by means of the spectroscope. As a standard of comparison the dilution was taken which was just sufficient to cause the *D* line of the haemoglobin spectrum to disappear. Several of the early readings were confirmed by Prof. C. J. Martin, to whom I am much indebted for help in this and other matters. Further controls of the accuracy of these readings were effected by comparing the results obtained by this with those of the ordinary colorimetric method when a comparatively strong solution of haemoglobin was used. The discrepancy was found to be of small amount.

The quantity of blood in the washings, muscle juice and bone juice having thus been obtained, the sum of these plus the 25 c.c. originally withdrawn gave the total blood volume. The details in the control experiments were:

Cats (normal).		Weight (grams)	Blood vol. (c.c.)	Vol./Wt. (%)	Percentage washed out
A.		4200	189.5	4.70	96.2
B.		1990	98.9	4.97	97.3
C.		2415	116.9	5.07	96.6
Average		—	—	4.91	96.7
Dogs (normal).		Weight (grams)	Blood vol. (c.c.)	Vol./Wt. (%)	Percentage washed out
Z.		—	1130.2	—	92.9
E.		5400	525.2	9.72	98.1
F.		11000	1120.4	10.19	92.5
X.		9100	545.6	6.00	91.6
Y.		9800	744.3	7.59	96.0
Average		—	—	8.87	94.2

The complete Welcker method is a tedious process and one which in the case of a large animal could with difficulty be completed in a day. On that account it was decided to take the amount of blood washed out as a constant percentage (94% of the total volume). In the remaining experiments therefore only the amount of blood washed out was estimated and the volume calculated therefrom.

Experimental Series.

I do not propose to enter here into the full details of each experiment, which would involve endless repetition and be of little interest. Only the outstanding data will be given, with explanatory notes wherever necessary.

A. (Stock.)

Apr. 18th, 1910. Infection started. Wt. 5.9 kg. Hb. 106%. Erythrocytes 6.38 millions.
 May 5th. *Ancylostoma* ova in faeces. Wt. 6.4 kg.
 June 7th. Infection stopped.
 July 19th. Wt. 5.8 kg. Hb. 76%.
 Oct. 7th. Hb. 96%. Red cells 5.99 millions. Eosinophils 0%.
 „ 24th. Hb. 84%. Red cells 7.15.
 Apr. 20th, 1912. Alive and well. Several ova in faeces.
 June 22nd. Killed. Hb. 96%. Red cells 5.56. Wt. 8.3 kg. Blood volume 632 c.c.
 = 7.62% of body-weight.

B.

Apr. 19th. Infection started.
 „ 24th. Dead. Pneumonia. No hook-worms in intestine.

C.

Apr. 20th. Infection started.
 May 5th. Dead. Pneumonia. Large number of immature hook-worms in ileum.
 Considerable amount of blood in intestine.

D. (Stock.)

May 11th.	Infection started. Wt. 9.1 kg. Hb. 90 %.	Red cells 5.63.
June 13th.	Ova in faeces. Infection stopped.	
July 1st.	Hb. 79 %.	
Oct. 7th.	Hb. 77 %.	Eosinophils 0 %.
Apr. 20th, 1912.	Alive and well. Moderate number of ova in faeces.	

G.

Feb. 28th, 1911.	Infection started.	
Mar. 4th.	Dog ill.	
„ 9th.	Very ill.	
„ 16th.	Dead. P.M. no obvious lesions. 12 immature hook-worms in intestine.	
	Not much blood.	

H.

Mar. 2nd, 1911.	Infection started.	
„ 9th.	Very ill.	
„ 14th.	Dead. Pneumonia. 12 young hook-worms in intestine; also a few round-worms. Large amount of haemorrhage.	

I.

Apr. 5th, 1911.	Infection started. Wt. 13 kg. Hb. 92 %.	Red cells 6.35.
May 2nd.	Wt. 12.6. Hb. 68 %.	Eosinophils 3½ %.
„ 21st.	Wt. 12.2. Hb. 77 %.	Eosinophils 1 %.
July 10th.	Wt. 12.3. Hb. 79 %.	Eosinophils 3 %.
Sept. 1st.	Wt. 12.7. Hb. 98 %.	Red cells 6.95. Eosinophils 0 %.
	Erythroblasts 650 c.mm.	
	Killed. Blood volume 905 c.c. = 7.13 % of body-weight. P.M.: viscera practically bloodless. 28 hook-worms in intestine. Very little blood in intestine, but large number of haemorrhagic areas of considerable size.	

J.

May 4th, 1911.	Infection started. Weight 8 kg. Hb. 88 %.	Eosinophils 12½ %.
	Already infected with <i>Ascaris</i> .	
„ 29th.	No ova in faeces. Wt. 9.2 kg. Hb. 88 %.	
June 6th.	Few ova in faeces.	
July 10th.	Wt. 9.4 kg. Hb. 59 %.	Eosinophils 2 %.
„ 12th.	Hb. 75 %.	Red cells 6.41. Eosinophils 5 %.
„ 15th.	Wt. 10 kg. Hb. 68 %.	Red cells 6.5. Eosinophils 5 %.
	Blood volume 729.3 c.c. = 7.29 % of body weight. P.M.: intestine contained a considerable amount of bloody mucus, with small clots of blood in places. About 170 specimens of hook-worm were met with from jejunum down to caecum, also 21 specimens of <i>Ascaris</i> in jejunum.	

K.

May 17th.	Infection started. Wt. 15.1 kg. Eosinophils 3 %.	
„ 18th.	Hb. 88 %.	Eosinophils 3 %.
„ 29th.	Wt. 11.7 kg. Hb. 81 %.	
June 11th.	Ova in faeces.	
July 10th.	Infection stopped.	
„ 14th.	Wt. 15.8 kg. Hb. 101 %.	Red cells 6.90. Numerous ova in faeces.

Aug. 7th.	Infection restarted.
„ 21st.	Wt. 14.5 kg. Hb. 97 % ₁₀₀ . Eosinophils 0 % ₁₀₀ . Red cells 8.5.
Sept. 7th.	Infection stopped again.
„ 29th.	Wt. 17.5 kg. Hb. 97 % ₁₀₀ . Red cells 8.7.
Oct. 20th.	Wt. 17.1 kg. Hb. 105 % ₁₀₀ . Eosinophils 0 % ₁₀₀ .
	Killed. Blood volume 1878.4 c.c.=10.98 % ₁₀₀ of body wt. P.M.: some injection in mesentery. Large amount of bright red blood in rectum and patches throughout the intestine, with numerous haemorrhagic points. 146 specimens of hook-worm dispersed throughout whole length of intestine, 9 being found in the rectum and 3 in the caecum. All were adult and many were full of blood.

L.

May 18th, 1911.	Infection started. Wt. 6.6 kg. Hb. 95 % ₁₀₀ . Eosinophils 4 % ₁₀₀ .
June 11th.	Numerous ova in faeces.
„ 20th.	Weak. Wt. 5.6 kg. Hb. 53 % ₁₀₀ . Eosinophils 15½ % ₁₀₀ .
July 10th.	Hb. 55 % ₁₀₀ . Red cells 5.94. Eosinophils 6 % ₁₀₀ .
„ 11th.	Wt. 6.3 kg. Hb. 51 % ₁₀₀ . Red cells 5.98. Eosinophils 3 % ₁₀₀ .
Aug. 21st.	Wt. 7.9 kg. Hb. 99 % ₁₀₀ . Red cells 8.02. Eosinophils 0 % ₁₀₀ .
Sept. 7th.	Infection stopped.
Oct. 3rd.	Wt. 7.0 kg. Hb. 93 % ₁₀₀ . Red cells 5.83. Eosinophils 1 % ₁₀₀ .
„ 26th.	Wt. 7.1 kg. Killed.
	Blood volume 427.2 c.c.=6.02 % ₁₀₀ of body weight. P.M.: intestine full of blood. 130 hook-worms, including several in caecum and rectum.

M.

May 22nd.	Infection started. Wt. 9.4 kg. Hb. 104 % ₁₀₀ . Eosinophils 3 % ₁₀₀ .
June 15th.	Ova in the faeces.
July 14th.	Wt. 8.9 kg. Hb. 81 % ₁₀₀ .
Sept. 7th.	Infection stopped.
Oct. 5th.	Wt. 8.1 kg. Hb. 95 % ₁₀₀ . Red cells 7.04. Eosinophils 0 % ₁₀₀ .
Dec. 5th.	Wt. 8.5 kg. Hb. 98 % ₁₀₀ . Killed.
	Blood volume 769.5 c.c.=9.05 % ₁₀₀ of body weight. P.M.: very little blood or signs of haemorrhage. 30 hook-worms in intestine.

O.

Aug. 3rd.	Infection started. Wt. 8.3 kg.
„ 17th.	Wt. 7.6 kg. Hb. 86 % ₁₀₀ . Red cells 5.0. Eosinophils 0 % ₁₀₀ .
„ 24th.	Very ill. Infection stopped.
„ 30th.	Dead. Wt. 5.3 kg.
	P.M.: very much emaciated. Mesentery greatly injected. Lower part of intestine, caecum and rectum of dark green colour from diffused blood. Intestine contained a small amount of blood. Other organs normal. 318 hook-worms found in intestine. In trachea were found two living <i>Agchylostoma</i> larvae (62 mm. in length), one was found in oesophagus and five in stomach, measuring .66-.74 mm. in length; also one small immature female, measuring 3.1 mm., in the stomach.

P.

Aug. 9th.	Infection started. Wt. 5.5 kg.
„ 16th.	Hb. 68 % ₁₀₀ . Red cells 4.07. Eosinophils 1 % ₁₀₀ . Ill and weak.
„ 24th.	Very ill. Infection stopped. No ova in faeces.
„ 25th.	Dead. Wt. 4.4 kg.

P.M.: much emaciated. Mesentery greatly injected. Viscera very pale. Lower part of intestine and rectum of dark green colour and containing a large quantity of blood. In intestine were found 1886 hook-worms, 12 specimens of *Dipylidium caninum*, and one *Ascaris*. No larvae in luugs or trachea.

Q.

Sept. 26th.

Infection started. Wt. 7·8 kg.

Nov. 9th.

Killed. Wt. 7·8 kg. Hb. 108 $\frac{1}{10}$.

Blood volume 493·1 c.c. = 6·49 $\frac{1}{10}$ of body weight. P.M.: only two hook-worms found in intestine. No blood.

S.

Jan. 20th, 1912.

Wt. 3·0 kg.

Feb. 23rd.

Wt. 5·0 kg. Hb. 70 $\frac{1}{10}$. Eosinophils $\frac{1}{2}$ $\frac{1}{10}$.

Apr. 9th.

Wt. 9·0 kg.

May 3rd.

Wt. 6·5 kg. Hb. 86 $\frac{1}{10}$. Red cells 6·32. Whites 15·0. Eosinophils 4 $\frac{1}{10}$.

„ 7th.

Wt. 6·8 kg. Hb. 76 $\frac{1}{10}$. Red cells 5·09. Whites 12·3. Eosinophils 2 $\frac{1}{10}$.

„ 10th.

Wt. 6·9 kg. Hb. 80 $\frac{1}{10}$. Red cells 5·10. Whites 15·8.

„ 13th.

Wt. 7·1 kg. Hb. 82 $\frac{1}{10}$. Red cells 6·19. Eosinophils 1 $\frac{1}{10}$.

Infected with *Ankylostoma* larvae by the mouth.

„ 14th.

Wt. 7·7 kg.

„ 16th.

Wt. 7·6 kg. Hb. 72 $\frac{1}{10}$. Red cells 5·15. Whites 15·7. Eosinophils 0 $\frac{1}{10}$. Erythroblasts 235.

„ 18th.

Wt. 7·4 kg. Hb. 72 $\frac{1}{10}$. Red cells 5·69.

„ 22nd.

Wt. 8·3 kg. Hb. 74 $\frac{1}{10}$. Red cells 6·50. Whites 20·1. Eosinophils 1 $\frac{1}{10}$. Erythroblasts 502.

„ 25th.

Wt. 8·8 kg. Hb. 74 $\frac{1}{10}$. Red cells 5·79. Whites 11·8. Eosinophils 3 $\frac{1}{10}$. Erythroblasts 1121.

„ 28th.

Wt. 8·9 kg. Hb. 76 $\frac{1}{10}$. Red cells 6·17. Whites 15·2. Eosinophils 6 $\frac{1}{2}$ $\frac{1}{10}$. Erythroblasts 304.

„ 29th.

Wt. 9·0 kg. Hb. 72 $\frac{1}{10}$. Red cells 5·90. Eosinophils 10 $\frac{1}{2}$ $\frac{1}{10}$. Erythroblasts 1292.

June 1st.

Wt. 9·2 kg. Whites 18·4. Eosinophils 2 $\frac{1}{10}$. Erythroblasts 11,960.

„ 3rd.

Wt. 8·8 kg. Hb. 60 $\frac{1}{10}$. Red cells 4·89. Whites 9·5.

„ 6th.

Wt. 8·2 kg. Hb. 52 $\frac{1}{10}$. Red cells 3·32. Whites 14·0.

„ 7th.

Wt. 8·1 kg. Hb. 56 $\frac{1}{10}$. Red cells 3·57. Whites 18·8. Eosinophils 2 $\frac{1}{2}$ $\frac{1}{10}$. Erythroblasts 3102.

„ 9th.

Wt. 7·3 kg. Hb. 52 $\frac{1}{10}$. Red cells 4·05. Whites 18·7. Eosinophils 6 $\frac{1}{2}$ $\frac{1}{10}$. Erythroblasts 654. Temp. 98·8. Very ill. Thin and feeble. No bleeding and apparently no pain or tenderness.

„ 10th.

Wt. 7·0 kg. Hb. 50 $\frac{1}{10}$. Red cells 3·77.

„ 11th.

Wt. 6·8 kg. Hb. 50 $\frac{1}{10}$. Red cells 3·29. Whites 22·7. Eosinophils 2 $\frac{1}{10}$. Erythroblasts 12,031.

„ 14th.

Wt. 6·5 kg. Hb. 48 $\frac{1}{10}$. Red cells 3·16. Whites 19·5. Eosinophils 4 $\frac{1}{10}$. Erythroblasts 1462.

„ 15th.

Wt. 6·8 kg. Hb. 48 $\frac{1}{10}$. Red cells 2·97.

„ 18th.

Wt. 6·6 kg. Hb. 40 $\frac{1}{10}$. Red cells 4·17. Whites 20·8.

Killed. Blood volume 490 c.c. and 7·43 $\frac{1}{10}$. 791 hook-worms in intestine, which contained considerable quantity of blood.

T.

May 7th, 1912.	Wt. 4.3 kg. Hb. 78 $\frac{9}{10}$. Red cells 5.97. Whites 7.7. Eosinophils 2 $\frac{9}{10}$. Erythroblasts 422. Temp. 102.2. Already infected with <i>Ascaris</i> .
„ 10th.	Wt. 4.4 kg. Eosinophils 0. Erythroblasts 38. Temp. 102.4° F.
„ 14th.	Wt. 4.4 kg. Hb. 78 $\frac{9}{10}$. Red cells 5.67. Whites 11.9. Eosinophils 1 $\frac{9}{10}$. Erythroblasts 0. Temp. 101.6° F.
„ 16th.	Wt. 4.2 kg. Hb. 82 $\frac{9}{10}$. Red cells 5.55. Whites 7.0. Eosinophils 0 $\frac{9}{10}$. Temp. 101.8.
„ 19th.	Wt. 4.5 kg. Hb. 88 $\frac{9}{10}$. Red cells 6.34. Whites 8.9. Eosinophils 4 $\frac{9}{10}$. Erythroblasts 177. Temp. 101.3–102.7° F.
„ 22nd.	Wt. 4.9 kg. Infection started. Fed by mouth.
„ 24th.	Wt. 4.9 kg. Hb. 78 $\frac{9}{10}$. Red cells 5.49. Eosinophils 0 $\frac{9}{10}$. Erythroblasts 1327.
„ 25th.	Wt. 5.0 kg. Hb. 70 $\frac{9}{10}$. Red cells 5.36. Whites 12.4. Eosinophils 7 $\frac{9}{10}$. Erythroblasts 3968. Temp. 102° F.
„ 29th.	Wt. 5.4 kg. Hb. 62 $\frac{9}{10}$. Red cells 4.42. Temp. 101.5° F. Eosinophils 3 $\frac{9}{10}$.
„ 31st.	Wt. 5.1 kg. Hb. 56 $\frac{9}{10}$. Red cells 3.98.
June 1st.	Wt. 5.0 kg. Hb. 52 $\frac{9}{10}$. Red cells 3.71. Temp. 101.8° F. Pulse 100.
„ 3rd.	Wt. 4.7 kg. Hb. 16 $\frac{9}{10}$. Red cells 90. Whites 19.7. Eosinophils 1 $\frac{9}{10}$. Erythroblasts 12,903. Temp. 98.8° F. Pulse 182. No ova in faeces. Very ill.
„ 4th.	Wt. 4.6 kg. Hb. 12 $\frac{9}{10}$. Red cells .88. Whites 20.7. Pulse 104. Temp. 94.4° F.
	Killed. Blood volume 293.7 c.c. = 6.38 $\frac{9}{10}$. 1719 hook-worms in intestine. Very large amount of blood in ileum, much of it bright red; also in caecum and large intestine. Several punched out pits in caecum. No larvae in trachea or oesophagus, but one found in stomach.

U.

Aug. 8, 1912.	Wt. 2.2 kg. Hb. 70 $\frac{9}{10}$. Red cells 5.18. Whites 21.1. Temp. 100.9° F. Already infected with <i>Ascaris</i> . Infected by mouth with hook-worm larvae.
„ 9.	Wt. 2.2 kg. Hb. 70 $\frac{9}{10}$. Red cells 5.75. Eosinophils 2 $\frac{1}{2}$ $\frac{9}{10}$. Erythroblasts 1793. No polychromasia. Temp. 101.3° F.
„ 10.	Wt. 2.2 kg. Whites 13.1. Eosinophils 2 $\frac{9}{10}$. Erythroblasts 1831. Temp. 101.8° F.
„ 12.	Wt. 2.3 kg. Hb. 75 $\frac{9}{10}$. Red cells 7.11. Temp. 100.8° F.
„ 13.	Wt. 2.5 kg. Whites 11.1. Eosinophils $\frac{1}{2}$ $\frac{9}{10}$. Erythroblasts 55.
„ 15.	Wt. 2.9 kg. Hb. 68 $\frac{9}{10}$. Red cells 6.56. Temp. 102.4° F.
„ 16.	Wt. 2.9 kg. Whites 18.4. Temp. 102° F.
„ 19.	Wt. 3.0 kg. Hb. 65 $\frac{9}{10}$. Red cells 7.72. Temp. 102.2° F.
„ 20.	Wt. 2.9 kg. Whites 15.8. Eosinophils 0 $\frac{9}{10}$. Erythroblasts 553. Temp. 101.6° F.
„ 21.	Wt. 3.1 kg. Whites 14.4. Eosinophils 0 $\frac{9}{10}$. Erythroblasts 720. Temp. 102° F.
„ 22.	Wt. 3.1 kg. Hb. 64 $\frac{9}{10}$. Red cells 8.35. Temp. 102° F.
„ 23.	Wt. 3.2 kg. Whites 11.4. Eosinophils 0 $\frac{9}{10}$. Temp. 101.4° F.
„ 29.	Wt. 3.6 kg. Hook-worm ova in faeces.
Oct. 15.	Wt. 5.8 kg. Still alive and well. No appearance of anaemia. Experiment discontinued.

V.

Aug. 8, 1912.	Wt. 2.0 kg. Hb. 70 $\frac{0}{10}$. Red cells 5.40. Whites 21.5. Eosinophils 3 $\frac{0}{10}$. Erythroblasts 430. Temp. 101.2° F. Already infected with <i>Ascaris</i> . Infected with hook-worm larvae through skin.
" 10.	Wt. 2.0 kg. Hb. 68 $\frac{0}{10}$. Red cells 6.02. Temp. 102° F.
" 12.	Wt. 2.0 kg. Whites 25.9. Eosinophils 3 $\frac{0}{10}$. Erythroblasts 388. Temp. 100.4° F.
" 13.	Wt. 2.2 kg. Hb. 70 $\frac{0}{10}$. Red cells 6.86. Temp. 101.3° F.
" 15.	Wt. 2.5 kg. Whites 15.6. Eosinophils 0 $\frac{0}{10}$. Temp. 102.2° F.
" 16.	Wt. 2.5 kg. Hb. 66 $\frac{0}{10}$. Red cells 7.56. Temp. 102° F.
" 19.	Wt. 2.6 kg. Whites 19.7. Eosinophils 0 $\frac{0}{10}$. Erythroblasts 197. Temp. 102.3° F.
" 20.	Wt. 2.6 kg. Hb. 61 $\frac{0}{10}$. Red cells 6.99. Temp. 101.2° F.
" 21.	Wt. 2.9 kg. Hb. 66 $\frac{0}{10}$. Red cells 6.43. Temp. 102.2° F.
" 22.	Wt. 2.8 kg. Whites 14.7. Eosinophils 0 $\frac{0}{10}$. Erythroblasts 147. Temp. 101.3° F.
" 23.	Wt. 2.9 kg. Hb. 65 $\frac{0}{10}$. Red cells 6.58. Temp. 102° F.
" 29.	Wt. 3.0 kg. Hook-worm ova in faeces.
Oct. 15.	Wt. 4.8 kg. Still alive and well. No appearance of anaemia. Experiment discontinued.

*General remarks with regard to time of appearance
of ova in faeces etc.*

A dog which first received larvae on April 18th, 1910, showed numerous ova in its faeces on May 5th, *i.e.* 17 days afterwards. This period corresponds with that found by other observers *e.g.* Lambinet (1905), Looss (1911), who have experimented with the dog hook-worm. A second dog, infected on April 19th, died on April 24th from pneumonia. No worms or larvae were found in the intestine. A third dog, infected on April 25th, died on May 5th from the same cause. Over 100 specimens of *Agchylostoma caninum* were found in the ileum. They were adult but none of the females contained mature ova. There had been a considerable amount of haemorrhage in the intestine, bright red blood being found as far down as the caecum. The death of these two dogs was almost certainly not to be ascribed to the infection with *Agchylostoma* for two other dogs in the same batch died from pneumonia before infection was started. From the lungs of these dogs an organism of the *Pasteurella* group was isolated, but whether this was the cause of death or not was not determined.

The first dog escaped the pneumonic infection, and small quantities of larvae were administered to it regularly every second day for about a month. It remained alive and healthy for over two years, and continued to show a moderate number of ova in its faeces during the

whole of that period. From it, chiefly, the remaining dogs of the series have been infected. At no time did it display any very marked signs of anaemia, although the haemoglobin percentage was at one time as low as 76. Blood examinations were made only at irregular intervals on this dog. On one occasion, during an attack of mange, the Hb percentage was as high as 96. Later, however, on treatment and recovery, it fell to 84. The erythrocytes rarely fell below six million per c.mm., while the leucocytes varied from 14-25 thousand, the higher figures being recorded during the early stages of infection. At no time was there any degree of eosinophilia, eosinophils being frequently entirely absent.

A fourth dog was infected on May 11th. No ova could be found in the faeces up to the 20th day. They were first seen on June 13th (33 days), but were probably present some days earlier as examination was intermitted for about a week. The weight of this dog, which was full grown, remained practically constant at about nine kilograms. The haemoglobin percentage, which was at first 95-100, had fallen to 80 when infection was established, *i.e.* when ova were demonstrable in the faeces, and remained about that figure for a year. The red cells did not fall below five millions per c.mm., while the leucocytes were generally about 20,000. In this case, again, no evidence of eosinophilia was obtained, but in the faeces there were distinct signs of intestinal haemorrhage. This dog is still alive and well and its faeces still contain moderate numbers of hook-worm ova. It has been maintained as a reserve stock.

As already remarked no pronounced symptoms of anaemia were observed in these dogs even after a month's constant infection. Similar infection was prolonged for periods of three, six, and more months in the case of other dogs, but in no case could a severe chronic anaemia, similar to that occurring in man, be produced. These dogs were all reputably under one year old and some were believed to be not much over six months. With still younger dogs there was the constant difficulty of intercurrent affections, such as pneumonia and distemper, to which in the majority of cases they succumbed soon after infection was started. These difficulties delayed the progress of the experiments very considerably, and it was at last decided to examine the blood volume of such dogs as had been infected and had shown signs of anaemia even in a minor degree. The general course of infection in these dogs was the advent of a slight though distinct degree of anaemia about three to four weeks after infection was started, this continuing for

varying periods of a month or longer, and being followed by gradual recovery, and this in spite of continued infection. The Hb percentage in some cases sank as low as 50, but the red cells were rarely under 5,000,000. The Hb index was therefore usually decidedly under unity. In the young dogs which did not succumb to intercurrent affections the symptoms were much more severe and the disease rapidly terminated in death. It is to be regretted that the blood volume of these dogs was not estimated, but this, unfortunately, was deferred in the hope of obtaining a chronic affection, a hope, however, which was in no case realised.

The results of the blood volume estimations may be tabulated as follows:

	Weight (gms.)	Blood washed out (c.c.)	Total vol. (calculated)	Blood vol. body wt. (%)	Hb %	No. of worms	Length of infection (days)
A.	8300	595.0	632.0	7.62	—	12	796
I.	12700	851.2	905.0	7.13	96	28	150
J.	10000	685.6	729.3	7.29	68	170	72
K.	17100	1766.5	1878.4	10.98	105	146	129
L.	7100	401.7	427.2	6.02	95	130	134
M.	8500	723.4	769.5	9.05	98	30	170
N.	9800	714.7	744.3	7.59	—	2	264
Q.	7800	463.5	493.1	6.49	108	2	44
S.	6600	461.0	490.4	7.43	40	791	36
T.	4600	276.1	293.7	6.38	12	1719	13
Aver.	9230			7.60 (1/13)			

On comparing this table with that on page 379 it is evident that on the average the blood volume per kilog. is distinctly less in the infected dogs than in the normal, although at the same time it must be noted that the infected dogs were on the whole a somewhat heavier lot.

Calculating the blood volume according to the formula of Dreyer and Ray, and taking as the observed volume only the amount washed out, we obtain the following values of the constant "k."

Normal	Infected
E. 0.746	I. 0.638
F. 0.873	J. 0.672
X. 0.599	K. 0.386
Y. 0.477	L. 0.922
	M. 0.572
	N. 0.642
	Q. 0.836
	S. 0.763
	T. 0.927
Average 0.674	0.706

From this it would appear that if the blood volume is a function of the surface and not of the body weight there is on the average little difference between the infected dogs and the normal, but that if anything the volume is somewhat decreased; individually there are three very marked deviations, namely in the case of dogs *K* and *L* and *T*, the former of which has a decidedly increased volume and the latter a markedly diminished volume.

The oxygen capacity of the blood per kilo of body weight has also been calculated, in this case on the estimated total volume, and the figures are as follows:

Normal:—*E.* 14.6. *F.* 10.8. *X.* 19.0. *Y.* 16.7. Average 15.3.

Infected:—*I.* 12.1. *J.* 9.4. *K.* 21.9. *L.* 10.6. *M.* 16.4. *N.* 14.6. *Q.* 13.3.

S. 5.5. *T.* 2.4. Average 11.8.

From this we see that on the average the oxygen capacity per kilo of weight is diminished in the infected dogs, but that at the same time there is a considerable amount of variation even in the normal dogs. We notice the very high figure given by *K*, and the very low figures of *S* and *T*, in which there was massive infection and considerable bleeding into the intestine.

These results are so variable, even in the case of normal animals, that it is difficult to draw any very definite conclusions from them. One fact would appear, namely, that normal dogs show wide variation in the factors on which the results of these experiments are based and on that account it would be necessary to obtain some very wide deviations in order to arrive at any definite conclusion.

A further endeavour was made to deduce some relation between the change in blood volume and the number of worms present, and the length of infection, but as will be seen from the table above no constant relation is obvious.

There are two additional matters which must be discussed before attempting to draw any final conclusion. These are the absence of eosinophilia and the presence of erythroblasts. The diagnostic significance of eosinophilia has already been mentioned in the earlier part of this paper, and it is somewhat remarkable to find it so constantly absent in the animals under investigation. The only animals in which it was at any time noted were *J* and *L*. In the former it was present before infection was started, and from the fact that a large number of round worms were found in the intestine after death, it is not improbable that they had some connection with the eosinophilia. In the case of *L* the eosinophilia made its first appearance about a month after infection was

started, but it fell off considerably during the course of the succeeding month, and eventually disappeared. No ready explanation of this curious circumstance presents itself. It must serve, however, to emphasise the fact that eosinophilia is not by any means a constant or invariable accompaniment of infection with intestinal worms.

The marked occurrence of erythroblasts in some cases is also worthy of note. In the earlier of these observations no account was taken of these cells, and it is possible that they escaped notice. Latterly, however, they were looked for and their numbers estimated. They did not occur in every case, but were particularly noted in dogs *I* and *R*. In the former they were found in the very considerable number of 650 per c.mm. They were also noted, however, in the young uninfected pup *R* to the extent of 100 per c.mm. Later, after infection, the number rose to 300 and eventually to 1150 per c.mm., after which it again fell to 300. These cells were usually of the normoblastic type and no megaloblasts were seen. The majority had a central nucleus, an evidence of their recent formation, but all stages were seen up to those in which the nucleus was quite peripheral. No rosette-shaped or dividing nuclei were observed. To me it appears there can be little question that the presence of these cells gives evidence of a distinct and active regeneration of blood cells to meet, presumably, the loss by haemorrhage, and this lends support to the view that the intestinal haemorrhage plays some part in the causation of the anaemia, at any rate in the early part of the infection. The occurrence of erythroblasts in the young normal pup is not, I believe, an abnormal circumstance, as these cells are not infrequently observed in the blood of children and of young animals for a few months after birth.

Other features in regard to the leucocytes were noted during the course of these experiments, the most interesting of which was the occurrence of intense basophilia during attacks of mange.

In addition to these observations, others, of biological rather than clinical interest, may be mentioned. Some of these have already been remarked upon in the opening part of this paper. There is first of all the remarkable fact that while young dogs may be infected to an extent only limited by death, older dogs, on the other hand, appear to be susceptible only to a very restricted degree. This is a circumstance which led to very great delay in the present research. It was always found possible to infect dogs, irrespective of age, and in the course of two or three weeks ova were found in the faeces. It was a natural assumption that by continuing the administration of infective material,

a very gross infection would eventually supervene, but such, as has already been stated, did not prove to be the case. It is impossible to imagine what factor enabled them to withstand further infection and the problem is one which undoubtedly possesses considerable biological interest.

Of a similar nature were the facts derived from attempts to infect cats and monkeys with the dog hook-worm. The cat, as might be expected from zoological considerations, is to a certain extent susceptible to infection with parasites of the dog. Thus, we find the common tape-worm, *Dipylidium caninum*, as frequent in cats as in dogs. The round-worms ("*Ascaris canis*" and "*Ascaris mystax*") also appear, to a certain extent, to be common to both, although this is a matter for further and more accurate investigation. On the other hand, the tape-worms of the genus *Taenia* appear to be extremely specific. The cat has its own species, *T. crassicolis*, which is never found in the dog, and conversely, the numerous species of *Taenia*, met with in the dog, are never found in the cat. The hook-worms appear to occupy an intermediate position as regards specificity. One species, *Uncinaria crinitiformis*, has been recorded only from the dog, while *Alghylostoma caninum* has been recorded from both, although much more frequent in the dog.

I attempted a considerable number of infection experiments with kittens, about two to four months old, but the results were vitiated by the "distemper" which almost invariably followed. This "distemper" occurred at two well-marked periods, namely, about the third and sixth months. In a litter of half a dozen, half would succumb at the third month, the others recovering only to have a second attack at the sixth month, from which there would be a solitary survivor. The disease was usually of the broncho-pneumonic type but not infrequently accompanied by gastro-enteritis. In only three cases did the kittens live long enough to display hook-worm eggs in their faeces. Only one survived much beyond the sixth month and this animal continued to pass eggs for about six months, but at the end of nine months it had apparently got rid of infection for no eggs could be detected after this period. Of two adult cats, one became slightly infected, the other was absolutely insusceptible.

Similar experiments were tried with three monkeys (*Macacus*) but in no case did infection take place, although they were fed with infective material continuously for three months.

Finally the experiment was tried of personal infection, but this also

was unsuccessful, and although infective cultures have been handled both by myself and by my assistant very frequently during the last two years, without any stringent precaution, no infection has taken place in either. This fact lends support to the belief that the dog hook-worm cannot infect man, and disposes of the idea that the dog can act as the carrier of hook-worm infection in man, either by harbouring the human form or by spreading its own particular species.

Owing to my departure for Australia these experiments have had to be discontinued and some of the points which might have required further investigation have had to be left incomplete.

SUMMARY AND CONCLUSIONS.

In these experiments the hook-worm anaemia of dogs does not appear to be exactly analogous to the corresponding disease in man, but differs from it in two essential particulars, namely, that only young animals suffer and that in them its course progresses much more rapidly to a fatal termination.

Older dogs, although not altogether insusceptible, acquire infection only to a moderate extent, which gives rise to a minor degree of anaemia. From this they gradually recover, even in spite of repeated and continued attempts at re-infection.

The anaemia in young dogs was characterised by great loss of weight, emaciation, prostration and intestinal haemorrhage, but in no case was epistaxis observed.

The blood volume of dogs suffering from the minor degree of hook-worm anaemia is not materially altered, but if anything is somewhat diminished. The oxygen capacity of the blood per unit of body weight is also, on the average, somewhat decreased.

Infection is generally accompanied by distinct though not profuse haemorrhage, which is most marked in the early stages, but tends to disappear.

Eosinophilia was not a constant sign either of infection or of disease.

Evidence of blood regeneration was furnished by the appearance of large numbers of erythroblasts (normoblasts) which increased with the progress of the disease.

Cats are much less easily infected than dogs, and monkeys are altogether insusceptible. Man, also, were found to be insusceptible to infection with the dog hook-worm.

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NOTE ON THE *B. COLI* TEST.

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My friend Major Wesley Clemesha, I.M.S., in a recent contribution¹ to the *Journal of Hygiene* is good enough *inter alia* to say:

"It may be safely stated that anything which Dr A. C. Houston writes on the subject of water bacteriology is received with very great interest and respect, and his recent report on the bacteriological characters of *Bacillus coli* in raw, stored and filtered river water (*Metropolitan Water Board, 7th Report on Research Work*) is no exception to the rule. The report is of very great interest to all engaged in the study of water bacteriology, particularly in England."

In attempting, however, to answer some of Clemesha's criticisms, it will be understood that I do so in a spirit the reverse of hostile. My position coincides with my inclination, and precludes me from entering the arena of controversy. Destructive criticism is nearly always to be strongly deprecated, and any remarks that I have to make are offered from the point of view of the constructive critic who is anxious to clear up any misconceptions and to bring into harmony views which may be apparently rather than really divergent.

The following quotations are taken from pages 463-465 of Clemesha's paper.

¹ Clemesha, W. W. (10. i. 1913), "A Criticism of Dr A. C. Houston's Report on the Biological Characters of *B. coli* Isolated from (1) Raw, (2) Stored River Water, (3) Stored and Filtered Water." *Journal of Hygiene*, xii. pp. 463-478.

Note.—To assist the reader, quotations from Clemesha's paper are printed in ordinary type; quotations from my own reports appear in smaller type; both are indented.

Dr Houston commences his report as follows:

"For many years past I have pointed out the striking differences between the varieties of *B. coli* found in excremental matter and in waters of diverse origin. As regards the Metropolitan water supply, it has been shown that the proportion of the *B. coli* organisms which ferment lactose with gas production (lactose +) and also produce indol in peptone cultures (indol +) as compared with those which fail in one or other or both of these respects, is as follows:

Water	Samples	Specimens (Glucose+)	Typical "Lactose+ Indol+" (per cent.)	Non-typical "Lactose - Indol+," or "Indol - Lactose+" (per cent.)
Raw River Waters	3,546	12,744	81.2	18.7
Filtered Waters	35,696	18,960	50.8	49.1

"These results have always been, and are now again, given simply as facts observed, when working with liquid primary media and not, as in any way, necessarily representing the actual ratio of typical to non-typical *B. coli* in the water originally.

"These findings have resulted from the adding of 100 c.c., 10 c.c., 1 c.c. and lesser amounts of water to one or other liquid primary medium, incubating at or about blood heat, usually for forty-four hours, planting out the colonies on one or another solid medium, and picking off the suitable colonies, and studying their biological attributes in a variety of culture media.

"Two points are specially to be remembered in connection with the use of liquid primary media. (1) The original proportion between the various kinds of *B. coli* may be greatly altered by the multiplication process. Indeed, the method aims at encouraging the typical *B. coli* to multiply and oust the non-typical *B. coli* in the struggle for existence in a liquid nutrient medium. (2) The specimens of *B. coli* may and do pertain to different volumes of the water being tested."

Clemesha goes on to say:

"In the quotation given above Dr Houston explicitly says that the figures obtained do not necessarily represent the actual ratio of the two kinds of bacilli in the original water. Now supposing that it can be definitely proved that there is a very great difference in the ratio of the classes between the actual water and these results, surely it must be admitted that the method is unsatisfactory. In other words, any method which can be proved to give a really reliable picture of the flora actually present in the water sample, must be superior, from the point of view of the water analyst, to one which cannot be relied on to do this. The main object of all analyses of water is to obtain an accurate picture of the actual bacteriological state of affairs in the sample at the time, so that if

'the original proportion of the various kinds of coli may be greatly altered by the multiplication process,'

the utility of this particular method appears to us to be very questionable. For many years Dr Houston has been using this 'enrichment' method and presumably has been basing his opinion of the quality of water on its results; probably most water analysts besides ourselves believe that, when using a method very similar to his, they are getting a reasonably accurate picture of the flora of the sample in question so that it comes rather as a shock to us in India to read that Houston now says that this is not the case.

Dr Houston not only says that

'the original proportion between the various kinds of *Bacillus coli* may be greatly altered by the multiplication process' (in broth media) but "the method aims at encouraging the typical *Bacillus coli* to multiply and oust the non-typical in the struggle for existence in a liquid nutrient medium.'

In the first place, we wish to say that, within certain limits, we very much doubt the accuracy of both these statements, and, secondly, we should like to know on what experimental evidence this favouring of one variety of organism at the expense of others is actually based. No evidence of this kind is given in the report."

It is to be noted that I did not say that the original proportions between the various kinds of *B. coli* "is" greatly altered by the multiplication process, but "may be" greatly altered, etc. Also the words "do not necessarily represent, etc." were employed purposely in place of the words "do not represent," etc.

Is not Clemesha going a little too far when he says:

"The main object of all analyses of water is to obtain an accurate picture of the actual bacteriological state of affairs in the sample at the time, so that if 'the original proportion of the various kind of coli may be greatly altered by the multiplication process' the utility of this particular method appears to us to be very questionable."

Personally, I think that we obtain the truest hygienic picture, by purposely using media and methods, which do in effect tend to alter the (initial) proportion of microbes in favour of those whose presence we have come to regard as specially significant of undesirable pollution. I trust the shock to my friends in India will be mitigated when I

assure them that I still remain a firm believer in the "enrichment" method, for the very reason that I believe it tends to ensure the subordination of the bacteria which occupy a secondary place, as indicators of potential danger, and the exaltation of those of more direct importance.

It may be of interest to look at the direct and indirect (enrichment) methods from the point of view of a person possessed of no special bacteriological knowledge.

I venture to think that the following analogy is a fair one:

It is sought to ascertain whether there are any fish in a fish pond, and if so what species are present, and their relative abundance.

The fish pond is divided up into a series of intercommunicating compartments of equal size which can if necessary be isolated from each other for draining or cleaning operations.

Direct Method.

We might select one of these compartments (corresponding, let us say, to our 1 c.c. cultures), drain it dry, and exhaustively count the fish. Imagine that we found a total of 100 fish made up of 5 Chubb, 15 Carp, 20 Bream and 60 Roach.

On the assumption that our compartment was a truly representative one we should then be in a position to affirm absolutely that the pond contained:

Chubb	5%
Carp	15%
Bream	20%
Roach	60%

Indirect Method.

We might elect to run the water *plus* all the fish from one of the compartments into a separate pond altogether (enrichment medium) one containing to start with, no fish of any kind. After one or more years (corresponding to incubating our cultures for 18-48 hours) we might net a *relatively very small portion* of it, corresponding to the very minute quantity we spread over our plates in making cultures from our primary liquid enrichment medium.

Imagine that our net captured exactly 100 fish should we be privileged on an investigation of their "species" to say our original pond necessarily contained this, that, or the other fish in the same proportions.

I think not, because the conditions in the second pond might be favourable to certain species and unfavourable to others. In much the same way, it seems to me that the direct method is almost necessarily and fundamentally the standard by which we must judge all enrichment methods, which seek to represent the "actual ratio of typical and non-typical *B. coli* in the water originally."

There may sometimes be technical difficulties which render the direct method difficult, perhaps almost impracticable, but this does not really affect the principle involved. I am not arguing against the "enrichment" method or in favour of the "direct" method but merely trying to show that whilst one judges the hygienic quality of a water best by the former method, the latter method *does*¹ (whereas the former *may* not) give the actual ratios of different microbes in the water originally.

The following experiment will serve as an illustration.

Equal volumes of a sample of river Thames water were used to inoculate:

(A) Solid lactose bile salt peptone agar plates (direct method).

(B) Liquid lactose bile salt peptone tubes (indirect enrichment method).

After 24 hours incubation at 37° C. 50 colonies were sub-cultured from A.

After (a) 24 hours and (b) 48 hours incubation at 37° C. solid lactose bile salt peptone agar plates were made from B.

After 24 hours incubation at 37° C., colonies were picked off from the B plates ((a) and (b)) in the same way and in the same number as had previously been done in the case of (A).

The following table shows the results obtained:

	A Direct solid method	B Indirect liquid enrichment method	
		(a)	(b)
Glucose 0	15	4	1
Glucose +	29	11	3
Lactose 0			
Glucose +	6	35	46
Lactose +			
	50	50	50

¹ It is assumed, of course, that all the colonies growing on the direct plates are subcultured, and a similar number subcultured from the indirect plates, the same volume of water being used in each case, and in sufficient amount so as to be representative.

As for practical reasons only a proportion and not the whole of the colonies appearing on the *A* plates was subcultured it cannot be said that the results under *A* necessarily represent the whole of the facts. It seems to me, however, fairly obvious that the effect of the enrichment medium was to alter the initial ratios so as greatly to increase the relative number of lactose as compared with glucose fermenters.

This indeed is the chief merit of the enrichment process.

On page 468 Clemesha says:

"Again, if a mixture of two bacilli, such as *B. coli communis* Escherich (which is lactose + glucose +) and our bacillus *P* (which is glucose + lactose -) are mixed together in roughly equal proportions and inoculated into a glucose broth, it can be proved that within the first 24 hours there is little or no alteration in the relative numbers of the two classes; both bacilli fermenting glucose. But if the same mixture be inoculated into lactose broth, the lactose fermenters (*B. coli communis*) very rapidly overgrow the bacillus *P*, which does not ferment the sugar. Even in this case white colonies are usually found in the plate. As one would expect, the more bacilli *P* there are in the original mixture the less is the overgrowth of *B. coli* apparent, but in cases where the numbers are approximately equal at the time of inoculation, the *B. coli communis* undoubtedly very rapidly outgrows bacillus *P*. This is after all only what one would expect—a bacillus that does not ferment lactose would probably grow slower in a lactose medium than one that does—indeed it seems to us rather astonishing that a non-fermenter should grow so well in a sugar that it cannot alter in any way."

These remarks would seem indeed to lend support to my guarded statement that:

"These results have always been, and are now again, given simply as facts observed, when working with liquid primary media and not, as in any way, necessarily representing the actual ratio of typical to non-typical *B. coli* in the water originally."

Clemesha goes on to say:

"Another series of experiments has been carried out in order really to ascertain whether the picture obtained from subculturing an 18-hour broth culture is reasonably like the original substance used for inoculation. A small piece of faeces of either human

being or cow, was taken and was divided into two parts. One was carefully wiped over 4 or 5 bile salt neutral red lactose agar plates. The other was inoculated into a bile salt lactose broth, and was incubated for 18 to 20 hours (inoculation was usually made in the afternoon and subcultured on the following morning). A careful comparison between a large number of colonies obtained by both methods, shows that the results are practically identical, the species of coliform organisms and their numerical relation to each other correspond to a most wonderful degree. The more colonies that are identified in each case the greater is the resemblance between the two results. For our experiments we took 50 colonies from both and we carefully identified each organism.

It will be observed that these experiments are infinitely more conclusive than those quoted by Dr Houston making use of raw, stored and filtered water. The possibilities of error in centrifugation, precipitation, evaporation, filter-brushing, etc., are extremely great: whereas in our experiments making use of faeces, all these very doubtful factors are eliminated."

I believe the reason why the results by the direct and indirect methods corresponded so closely in the above experiment was because faeces and not water was used. Faeces contain typical *B. coli* in enormous numbers and I should have expected a closer parallelism between the results yielded by the two methods when working with this substance than with any other material. In the case of water the non-typical *B. coli* may greatly out-number the typical *B. coli* and this in my experience may lead to very different results being obtained.

Clemesha proceeds as follows:

"Dr Houston's method of water analysis and our own are almost identical in the first stage. For crude waters we inoculate both a lactose and a glucose broth with identical quantities of the sample, in order to find out the relative number of glucose and lactose fermenters present in the water. The full reasons for this procedure cannot be entered into here. For filtered waters we use a lactose broth only. Having done this, we take the tube that has received 20 c.c. of the sample, after 18 hours' growth in the incubator, and subculture the organisms present in this tube *only*. The various species of coliform organisms present are separated by MacConkey's method. We maintain that by this procedure we get

a very accurate picture of the true bacteriological flora of 20 c.c. of the water under analysis, and it is not necessary to separate species in smaller quantities. The actual number of lactose or glucose fermenters present in all smaller quantities is ascertained by the first step."

Personally, I prefer to make sub-cultures from *all* the tubes (100, 10, 1, .1, etc. c.c.) yielding positive presumptive results, but I am far from suggesting that Clemesha's modified procedure is not well suited for the purposes he has in view and the kind of waters he is called upon to examine.

I should have liked to have been able to test the matter with the London filtered waters, but happily for the consumers, they are relatively so good that a direct culture with 20 c.c. of water spread over 20 plates would (generally speaking) yield either no colonies at all or too few for subcultural purposes.

The following instructive experiment has however been carried out:

Direct Method.

(A) 5 c.c. of river Thames water were spread over five solid lactose bile salt peptone agar plates. The plates were incubated at 37° C. for 24 hours and then *all* the colonies (47 in number) were submitted to cultural tests.

Indirect Enrichment Method.

(B) 5 c.c. of the same sample of water were added to liquid lactose bile salt peptone water and incubated for 18 hours at 37° C. Solid lactose bile salt peptone agar plates were made therefrom and these were then incubated for 24 hours at 37° C.

A suitable representative plate was then taken and a section marked off with a coloured wax pencil, so as to embrace 47 unselected colonies and *all* of these were admitted to cultural tests.

The following results were obtained (p. 401).

It is difficult to see how any other conclusion can be reached than that the A (direct) method here shows as nearly as possible the absolute ratio of the microbes capable of growing on bile salt agar and pertaining to 5 c.c. of the sample of water, because *all* the colonies (47 in number) growing on the plates were subcultured. As regards the B (indirect) method it is obviously impossible to subculture the thousands or

Indol	Glucose	Lactose	Saccharose	Dulcite	A	B
					(Direct)	(Indirect)
0	0	0	0	0	35	5
0	+	0	0	0	1	0
0	+	0	+	+	1	0
+	+	+	+	+	1	0
0	+	0	+	0	2	0
+	+	+	0	0	3	7
+	+	0	0	0	1	20
0	+	+	+	0	2	1
+	+	+	+	0	1	9
+	0	0	0	0	0	4
0	+	+	0	0	0	1
Total					47	47

millions of microbes resulting from the multiplication of those originally present in the water, but it seems a fair means of comparison to map out a section of a plate so as to embrace 47 adjacent unselected colonies and subculture all the colonies within that area.

What is quite certain is this, that if *A* be regarded as the standard, *B* presents a highly distorted picture of the original state of the water. The results of this experiment, especially as they confirm my previous experience, seem to me fully to justify my use of the words, "The original proportion between the various kinds of *B. coli* may be greatly altered by the multiplication process." It is at once admitted that Clemesha by altering his procedure so as to shorten the period of incubation tends in the direction of preserving the initial ratios. On the other hand it must be remembered that this very circumstance may possibly weaken the value of the test from the hygienic point of view. Unless my memory is at fault, MacConkey, a good many years ago, advocated incubation for 48 hours for the very reason that it tended to alter the initial ratios and to bring into prominence those microbes specially significant of undesirable pollution. It is not however suggested that Clemesha is in any way wrong in adopting the particular methods and procedure which in his experience yield the most useful results in India.

On pages 470-472 Clemesha endeavours to show that the results obtained by me by the "direct" and "indirect" methods are strikingly similar. It would occupy too much space to go into this matter in detail, but I should welcome confirmation of such a conclusion, because my records by the "indirect" method are very numerous indeed whereas those obtained by the "direct" method are by *comparison* few in number.

From the tone of Clemesha's whole paper I rather gather that he thinks that my investigations by the "direct" method aimed at showing the imperfections of the "indirect" method. This, however, is far from being the case, my real object being to disarm criticism by using both methods, as otherwise it might be said that I had made no attempt to study the subject by the "direct" method.

Is not Clemesha a little severe when he criticises my statement :

"Lactose + indol + microbes are typical of excrement inasmuch as they are present therein in enormous number and are the predominant microbes in this material.

"Waters not recently contaminated with excremental matters contain none or very few of these bacteria."

as follows :

"To the second sentence we must take very grave exception. Waters are not infrequently met with in India, in lakes and storage reservoirs, which we know have received no pollution for probably two, three or even six months, yet the majority of these may contain as many as 1 to 10 lactose + indol + organisms per c.c."

Excremental matters usually contain 100,000 *B. coli* per gramme and even if a water contained 1 or even 10 *B. coli* per c.c. this is a very small number in comparison. A reference to my reports on storage, etc., would seem to justify my statement.

The remainder of Clemesha's paper is chiefly devoted to an expression of the desirability of differentiating between faecal organisms of separate species and applying the results obtained to the history of waters in relation to pollution. No one will quarrel with this view, and if some of us feel that the results so far obtained are in their practical bearing a little disappointing, it would be ungenerous not to applaud the work already done in this field of enquiry or to say anything likely to discourage the hope that work in the future will bear more ample fruit.

It is impossible, perhaps even undesirable, for all bacteriologists to see eye to eye with each other in considering and interpreting biological problems of considerable perplexity; but this need not debar us from displaying a tolerant attitude when dealing with the work of others. Otherwise we are apt, almost unconsciously, to slip backwards into the ranks of the mere destructive critic.

THE DETECTION OF ANTHRAX SPORES IN EAST INDIA WOOL AND IN YARN MANUFACTURED THEREFROM.

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THE following investigation was undertaken during November and December 1911 with the object of determining the source of infection in a series of cases of anthrax which occurred in the same yarn mill. The investigation resulted in proving that anthrax spores were present in a variety of East India wool and in the yarn manufactured therefrom.

Cases of Anthrax.

Case	Sex	Age	Occupation	Situation of pustule	Date
1. F. B.	Female	34	Condenser minder	Forearm	26. 5. 09
2. E. M.	"	18	Hanker	Face	18. 9. 09
3. A. H.	"	15	"	Arm	28. 6. 10
4. A. M. B.	"	21	"	Arm	7. 9. 11
5. A. S.	"	13	"	Elbow	27. 10. 11

The cases, five in number, occurred between May 26th, 1909, and October 27th, 1911, while one blend of wool was undergoing manipulation. With one exception they occurred among hankers, three of whom were employed at machines in the same room. In each case the diagnosis was confirmed by bacteriological examination. It seems remarkable that not a single case occurred among the workers engaged in the earlier and more dusty processes of shaking, blending and willeying.

Materials Examined and Result of Examination.

As the cases occurred chiefly among the hankers samples were in the first place obtained of the materials with which such workers would be most likely to come into close contact.

The specimens examined in the first instance consisted of :

Nos. 1 and 2. Specimens of wool dust from ring-twisters, taken from the machines in a room in which three cases of anthrax had occurred.

No. 3. Sample of unscoured yarn made from the same blend and identical with that on which the hankers were employed when the cases occurred.

No. 4. Wool dust and oil scraped up from the floor.

No. 5. Wool blend from which the yarn was spun.

The examination, details of which are given below, showed that anthrax spores were present in the sample of yarn No. 3. This yarn had undergone the complete process of manufacture except the final scouring in soap liquor. As far as could be ascertained this is the first recorded instance of a sample of manufactured wool having been found to contain anthrax spores.

The result of the examination of the other specimens was negative.

The following further specimens were next obtained and examined with negative result :

No. 6. Unscoured yarn (same source as No. 3).

No. 7. Scoured yarn (same source as No. 3).

No. 8. Soap liquor in which the hanks of finished yarn were scoured.

It was suspected that a particular variety of wool composing the blend might be infected and it was therefore decided to examine separately each class of wool employed in blending. The blend was composed of eight different varieties of wool which were as follows :

No. 9. Egyptian Wool 1. No. 13. East India Wool 5.

No. 10. East India „ 2. No. 14. East India „ 6.

No. 11. French „ 3. No. 15. Cape „ 7.

No. 12. East India „ 4. No. 16. East India „ 8.

Samples of each of these wools were examined and No. 14 East India Wool 6 was found to contain anthrax spores. In order to confirm this result a further sample No. 17, of East India Wool 6, was obtained from the same source. This was divided into three portions A, B, and C, each of which was examined separately. Portions A and C were found to contain the anthrax spores, but in the case of portion B the examination proved negative.

As two consecutive samples taken without selection gave positive results, it is probable that the spores were present in large numbers and were widely disseminated throughout this variety of wool. The

samples of wool were not obviously blood-stained, but most of them were contaminated with dried faecal material and all of them were very dusty.

The table shows the materials examined with the result of the examination in each case.

Method of Examination.

The specimens with the exception of No. 4 (wool dust and oil) and No. 8 (soap liquor) were placed in large flasks containing 250 c.c. to 500 c.c. of sterile saline solution. The quantity of solution, depending on the bulk of the sample, was in each case sufficient for complete immersion of the material. The flasks were thoroughly shaken and after standing 24 hours the whole of the washings were poured off and centrifuged. On centrifuging, a considerable quantity of compact brownish deposit collected at the bottom of the tube leaving a more or less clear supernatant fluid the bulk of which was withdrawn, only about 3 or 4 c.c. being allowed to remain. Slight agitation of the tube caused the upper layer of the deposit to mix with the remaining fluid forming an emulsion; from this emulsion cultures were made and guinea-pigs were inoculated.

Specimen No. 4 which consisted of masses of caked dirt, wool and oil was first ground up then thoroughly mixed with saline solution and centrifuged. The upper layer of the deposit was mixed with a little saline solution and examined.

Specimen No. 8 consisting of soap liquor in which the hanks of yarn were scoured, was centrifuged and the deposit obtained was repeatedly washed in saline solution to remove the soap. A small quantity of the washed deposit was examined.

Cultures.

Cultures were made on agar, two plates being used for each specimen. Several drops of emulsion were placed on the first plate and were spread over the surface by means of a platinum loop which was then rubbed over the surface of the second plate. In the case of specimen No. 17 portion C, no cultures were made. The anthrax bacillus was not obtained in any of the cultures. The isolation of the bacillus even although present was rendered impracticable owing to the presence of large numbers of organisms which produced colonies closely resembling, and sometimes almost identical with, those of anthrax. The organisms were probably the bacillus anthracoides of Bainbridge and

the varieties of anthrax-like organisms described by Page (1909) as occurring in abundance in wool and preventing the detection of anthrax colonies by their profuse growth.

Animal Inoculations.

In each case a guinea-pig was inoculated subcutaneously in the left thigh with one cubic centimetre of emulsion. Inoculations made from the following specimens gave positive results:

No. 3. Unscoured yarn.

No. 14. East India Wool (1st specimen).

No. 17. East India Wool (2nd specimen, portions A and C).

The guinea-pigs inoculated from these specimens died and all showed the typical post-mortem appearances of death from anthrax, viz. gelatinous oedematous exudate spreading subcutaneously from the seat of the inoculation over the abdominal wall; injection of the vessels and haemorrhages at the seat of inoculation; spleen dark in colour, enlarged, soft and friable; blood in the heart and great vessels dark in colour and fluid. The bacillus could be demonstrated microscopically in large numbers in the blood and tissues. Cultures were made on agar from the heart-blood, spleen and local exudate of each of the animals (except that inoculated with portion C of specimen No. 17) and abundant growths of anthrax bacilli were obtained.

The time elapsing between the date of inoculation and the death of the guinea-pig was longer than is usual after inoculation with pathological material obtained direct from cases of anthrax. One of the guinea-pigs died in three days, two in four days and one in six days. Glynn and Lewis (1912) found that the average duration of life in 40 animals dying from anthrax after inoculation with extracts of similar materials was 4.2 days and three survived 8, 7 and 7 days respectively. The average duration of life of guinea-pigs inoculated with materials from cases of anthrax is about two days. Of 26 animals recently inoculated in the West Riding Bacteriological Laboratory from cases, both human and bovine, 20 died within two days of inoculation and the remainder within three days.

The guinea-pigs inoculated from the following five specimens died of anaerobic infection.

No. 4. Wool dust and oil.

No. 6. Unscoured yarn.

No. 8. Soap liquor.

No. 12. East India Wool, 4.

No. 15. Cape Wool, 7.

Two of the guinea-pigs died in two days and three in four, three, and one day respectively.

The post-mortem appearances in some of these cases closely resembled anthrax, but instead of the gelatinous oedema the subcutaneous tissues were markedly bloodstained over the whole abdomen and thorax, the spleen was not enlarged or only very slightly so, and the blood in the heart and great vessels was clotted. Large Gram-positive bacilli were found in the exudate by microscopic examination, but were absent or only present in very scanty numbers in the heart-blood. No growth of these organisms was obtained on agar plates incubated aerobically and as they were Gram-positive they were probably either Welch's bacillus (*B. aerogenes capsulatus*) or the *B. enteritidis sporogenes* of Kleiu.

Webb and Duncan (1904) found, in a similar investigation, that in some cases the inoculated guinea-pigs died of malignant oedema before anthrax had time to develop and that cultures from the spleen of such animals on more than one occasion revealed the presence of anthrax bacilli. Glynn and Lewis (1912) were also able, in two instances, to isolate the anthrax bacillus by culture from the peritoneal fluid though the guinea-pigs had died of conditions other than anthrax, and these authors are of the opinion that the percentage of deaths from anthrax in their series would have been higher but for the presence of pathogenic anaerobes. In my own investigation cultures were made from the spleen, blood and exudate but no growth of anthrax bacilli could be obtained. In two cases a second guinea-pig was inoculated with emulsion of spleen, but only the original condition was reproduced and cultures gave negative results.

CONCLUSIONS.

1. Infected East India Wool was the cause of the occurrence of the cases.
2. For the detection of the *Bacillus anthracis* in material such as wool animal inoculations are necessary.
3. Guinea-pigs inoculated with wool washings containing anthrax spores tend to survive for a longer period than is usual after inoculation with material direct from cases of anthrax.

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WEBB and DUNCAN (1904) of Worcester. *County Council Report*, quoted by Page.
GLYNN and LEWIS (1912). The Detection of Anthrax Spores in Industrial Material. *Journ. of Hyg.* xii. 227--244.

Table of Specimens and Results of Examination.

No. and material	Result of cultures	Result of inoculations	Examination of animal tissues	Remarks
1. Wool dust...	<i>B. anthracis</i> not found	Negative. Guinea-pig remained well	—	—
2. Wool dust...	Do.	Do.	—	—
3. Unscoured Yarn	Do.	Negative. Guinea-pig died in 4 days. <i>Anthrax</i>	<i>B. anthracis</i> found by microscopical and cultural examination in very large numbers in the heart-blood, spleen and exudate	—
4. Dust and Oil	Do.	Negative. Guinea-pig died in 4 days. Anaerobic infection	<i>B. anthracis</i> not found	—
5. Blend Wool	Do.	Negative. Guinea-pig remained well	—	—
6. Unscoured Yarn	Do.	Negative. Guinea-pig died in 3 days. Anaerobic infection	<i>B. anthracis</i> not found	Second animal inoculated from spleen died in 1 day. <i>B. anthracis</i> not found.
7. Scoured Yarn	Do.	Negative. Guinea-pig remained well	—	—
8. Soap Liquor	Do.	Negative. Guinea-pig died in 2 days. Anaerobic infection	<i>B. anthracis</i> not found	Second animal inoculated from spleen died in 2 days. <i>B. anthracis</i> not found.
9. Egyptian Wool No. 1	Do.	Negative. Guinea-pig remained well	—	—
10. East India Wool No. 2	Do.	Negative. Guinea-pig remained well	—	—
11. French Wool No. 3	Do.	Negative. Guinea-pig remained well	—	—
12. East India Wool No. 4	Do.	Negative. Guinea-pig died in 2 days. Anaerobic infection	<i>B. anthracis</i> not found in the heart-blood, spleen and exudate	—
13. East India Wool No. 5	Do.	Negative. Guinea-pig remained well	—	—
14. East India Wool No. 6	Do.	Positive. Guinea-pig died in 6 days. <i>Anthrax</i>	<i>B. anthracis</i> found by microscopical and cultural examination in very large numbers in the heart-blood, spleen and exudate	—
15. Cape Wool No. 7	Do.	Negative. Guinea-pig died in 1 day. Anaerobic infection	<i>B. anthracis</i> not found	—
16. East India Wool No. 8	Do.	Negative. Guinea-pig remained well	—	—
17. East India Wool No. 6	Do.	—	—	—
(a) Portion A	Do.	Positive. Guinea-pig died, 3 days. <i>Anthrax</i>	<i>B. anthracis</i> found by microscopical and cultural examination in very large numbers in the heart-blood, spleen and exudate	—
(b) Portion B	Do.	Negative. Guinea-pig remained well	—	—
(c) Portion C	Do.	Positive. Guinea-pig died, 4 days. <i>Anthrax</i>	<i>B. anthracis</i> found by microscopical examination. No cultures made	—

THE IMMUNITY REACTIONS OF AN INAGGLUTINABLE STRAIN OF *B. TYPHOSUS*.

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(With 2 Charts.)

INAGGLUTINABLE strains of *B. typhosus* are of considerable interest to bacteriologists both from a practical and theoretical aspect. These strains present the usual characteristics of the typhoid bacillus, with the exception that they are not agglutinated by anti-typhoid sera, and if such a contingency be overlooked one may fail to recognise that one is dealing with a typhoid infection. This feature persists in the subcultures for many months, thus differentiating the inagglutinable strains from the many strains of *B. typhosus* which agglutinate with difficulty when freshly isolated but which regain their full agglutinability after one or two subcultures. Since their discovery these inagglutinable strains have been investigated on several occasions without any definite agreement as to the condition being arrived at.

The isolation of inagglutinable or feebly agglutinating strains of *B. typhosus* has been recorded by Achard and Bensaude (1896), Kolle (1897), Johnston and Taggart (1897), Van de Velde (1897), Sacquépée (1901), Remy (1901), Rodet (1902), Nicolle and Ternel (1902), Lipschütz (1904), Klinger (1902), Lesieur (1903) and others. Although a certain number of the above were obtained from chronic typhoid lesions, there is no evidence to show that there is any relation between inagglutinability and chronicity of infection. At present there is no record of the isolation of an inagglutinable strain from a "typhoid carrier." The strain on which the following observations were made was isolated from an acute case of typhoid fever.

Nicolle considers that there is a close relation between agglutination and motility, and the production of agglutinins. He worked with a non-motile strain which on injection into rabbits produced agglutinins neither for itself nor for the laboratory strain. On the other hand Remy states that inagglutinable strains readily produce agglutinins while Rodet doubts whether there is any relation between the agglutinogenic function and agglutinability. The latter found that an inagglutinable strain of *B. prodigiosus* was capable of producing on injection agglutinins for normal *B. prodigiosus*.

It would therefore appear that inagglutinability arises as the result of an increased resistance on the part of the bacilli to anti-bodies secreted against them. The artificial production of inagglutinable strains by Sacquépée, Walker (1902), and Müller (1903), support this view.

The inagglutinable strain, which forms the subject of this paper, was isolated from a patient whose clinical history was briefly as follows:

The patient was a boy (J. S.) aged 12 who had been out of sorts for a week or so previously to being seen on Sept. 30th, 1912. He was one of the earlier patients to be attacked in the recent Aberdeen epidemic of typhoid fever (1912-1913).

The clinical features of the case may be briefly summed up as being those of a continued fever with rapid pulse, showing symptoms and physical signs of capillary bronchitis with enlarged spleen. During the second week of high fever, diarrhoea set in with pea-soup stools. Deafness intervened during the third week but gradually disappeared during the ensuing weeks. The Widal reaction was first found to be positive on Nov. 4th, 1912. Previous tests had been negative while the blood also had failed to react to Paratyphoid B. We present a temperature chart of the case. On Oct. 16th a sample of blood was taken aseptically (10 c.c.) from the right median basilic vein and sown into sterile broth. The sample was incubated overnight at 37° C. A transplantation was made on to MacConkey bile salt agar plates and ultimately a bacillus was isolated from the blood with the cultural reactions of *B. typhosus*.

The patient's serum agglutinated the laboratory strain of *B. typhosus* in a dilution of 1 in 50 within five minutes, but failed altogether to agglutinate the bacillus recovered from the veno-puncture in a large series of dilutions. The infection was considered then a case of typhoid fever arising from an inagglutinable strain of *B. typhosus*.

Characters of the strain isolated from the case.

The organism isolated presented all the characteristics of *B. typhosus* with the single exception that it was not agglutinated by an anti-typhoid serum, as the following experiments show.

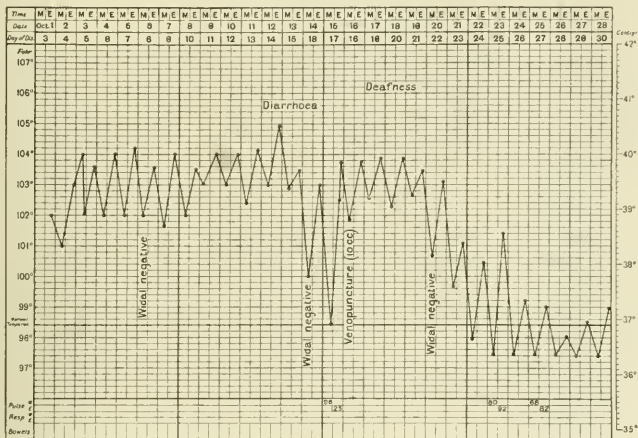


Chart 1.

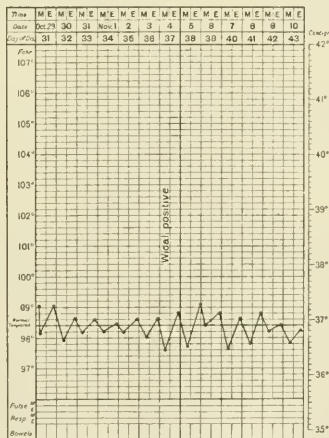


Chart 2.

Fermentation reactions.

The following table gives the fermentation reactions of the in-agglutinable typhoid bacillus. (Tested on five occasions.)

	Nov. 1 (24 hrs.)	Nov. 4	Nov. 5	Nov. 7	Nov. 14
Glucose	A	A	A	A	A
Lactose	—	—	—	—	—
Dulcitol	—	—	—	—	—
Saccharose	—	—	—	—	—
Mannite	A	A	A	A	A
Maltose	A	A	A	A	A
Dextrine	A ?	A	A	A	A
Galactose	A ?	A	A	A	A
Sorbitol	A	A	A	A	A
Inulin	—	—	—	—	—
Raffinose	—	—	—	—	—
Arabinose	—	—	—	—	—
Laevulose	A	A	A	A	A
Salicin	—	—	—	—	—
Erythritol	—	—	—	—	—
Milk	A ?				
Milk (first sub-culture)	A	A (pinkish colour, no clot)			

Subcultures made four months later showed no change in the fermentation reactions.

Agglutinability.

An anti-typhoid serum with a titre of about 1 in 5000 failed to agglutinate the bacillus in dilutions beyond 1 in 40, even after 24 hours.

*Experimental Part.**Agglutinogenic function.*

The problem that suggested itself to us was:—Could an inagglutinable strain of *B. typhosus* injected into an animal produce an anti-typhoid serum which would fail to agglutinate its own antigen yet agglutinate easily a normal strain of *B. typhosus*?

Injections were made into rabbits. The first injection consisted of an emulsion in sterile normal saline of half an agar slope culture (24 hours growth) of the inagglutinable *B. typhosus* killed by heating to 60° C. for 45 minutes. The inoculation was made intraperitoneally. The second injection made eight days later was similar but consisted of two agar slopes; the third injection made a week later consisted of

three agar slopes. Other two rabbits which were treated intravenously with an emulsion of the bacillus unfortunately died before their sera had acquired any great agglutinating power. Samples of blood were taken from the animals from time to time and tested. It was noted that the serum always failed to agglutinate its own antigen in a dilution of 1 in 50 and upwards, while an increasing capacity to agglutinate the normal *B. typhosus* was observed. Ultimately when the serum of a rabbit had acquired a titre of 1 in 1600 towards the normal typhoid bacillus, the animal was killed, the blood gathered aseptically, and the serum separated and stored.

The following tables give the reactions of the serum to its own antigen and to a normal agglutinating strain of *B. typhosus*. The emulsions of bacilli used in the test were prepared by emulsifying a 24 hours agar slope growth in normal saline or in carbolic saline (0.5 %) and the test carried out in small tubes.

The signs + and - denote positive and negative reactions respectively.

Macroscopic method.

B. typhosus (inagglutinable).

Serum dilutions	1-30	1-40	1-50	1-100	1-200	1-400	1-800	1-1600	1-3200
Agglut.	+	-	-	-	-	-	-	-	-

B. typhosus (agglutinating strain).

Serum dilutions	1-30	1-40	1-50	1-100	1-200	1-400	1-800	1-1600	1-3200
Agglut.	+	+	+	+	+	+	+	+	+

Microscopic method.

Tested by the hanging drop method where the criterion of clumping was taken to be the picture presented of obvious clumping under the low power of the microscope.

Reading after 30 mins.

B. typhosus (inagglutinable).

Serum dilutions	1-20	1-30	1-40	1-100	1-200	1-400	1-800	1-1600
Agglut.	+	-	-	-	-	-	-	-

B. typhosus (agglutinating strain).

Serum dilutions	1-20	1-30	1-50	1-100	1-200	1-400	1-800
Agglut.	+	+	+	+	+	+	+

Reading after 2 hours.

B. typhosus (inagglutinable).

Serum dilutions	1-20	1-30	1-40	1-100	1-200	1-400	1-800	1-1600
Agglut.	+	+	-	-	-	-	-	-

B. typhosus (agglutinating).

Serum dilutions	1-20	1-30	1-50	1-100	1-200	1-400	1-800
Agglut.	+	+	+	+	+	+	+

This serum therefore, prepared by the injection of the inagglutinable strain of *B. typhosus*, only agglutinates its own antigen in dilutions up to 1 in 30 in 24 hours by the macroscopic method, while the same serum can agglutinate the normal agglutinable strains of *B. typhosus* up to 1 in 1600. Consequently we may conclude that by the injection of the inagglutinable strain of typhoid bacilli an anti-serum can be produced which will not agglutinate its homologous strain but will agglutinate a heterologous agglutinable laboratory strain.

Accordingly an inagglutinable strain of *B. typhosus* can on injection elicit the production of agglutinin for agglutinable strains of the bacillus and not for itself; in other words the inagglutinable strain has agglutininogenic functions.

We then proceeded to determine the presence or absence of group agglutinins in the anti-serum.

B. coli.

Serum dilutions	1-50	1-100	1-200	1-400	1-800	1-1600	1-3200
Agglut.	-	-	-	-	-	-	-

B. paratyphosus (B).

Serum dilutions	1-50	1-100	1-200	1-400	1-800	1-1600	1-3200
Agglut.	-	-	-	-	-	-	-

B. gaertner.

Serum dilutions	1-40	1-80	1-160	1-320	1-640	1-1280	1-2560
Agglut.	+	+	+	+	-	-	-

B. paratyphosus (A).

Serum dilutions	1-25	1-50	1-100	1-200	1-400	1-800	1-1600	1-3200
Agglut.	-	-	-	-	-	-	-	-

B. dysenteriae (Flexner).

Serum dilutions	1-25	1-50	1-100	1-200	1-400	1-800	1-1600	1-3200
Agglut.	+	+	+	-	-	-	-	-

The injection therefore of an inagglutinable strain of typhoid into an animal produces an anti-serum which contains the group agglutinin usually present in an anti-typhoid serum. The peculiarity here is that the inagglutinable typhoid bacillus functioning as an antigen can produce a group agglutinin of higher specificity for a heterologous bacillus than for the homologous bacillus.

Inagglutinability.

It is now usually recognised that the mechanism of agglutination, as first pointed out by Bordet, consists of two stages (1) fixation, (2) aggregation. The question then presents itself: Does the inagglutinable strain of *B. typhosus* fix (absorb) typhoid agglutinin in a manner similar to that of the normal agglutinating typhoid bacillus?

(1) *Absorption.* The first investigation on this problem was made with the idea of ascertaining whether the agglutinin in the inagglutinable serum is capable of being absorbed by the inagglutinable strain.

Experiment 1. Equal quantities of the serum were measured out in sterile tubes and labelled "A" and "B."

To "A" four loops of culture of *B. typhosus* (inaggl.) were added and emulsified. To "B" no addition was made. Both were put in the incubator at 37° C. for two hours, then centrifugalised and equal quantities of the clear supernatant fluid pipetted off and dilutions made with sterile normal saline.

"A." Saturated with *B. typhosus* (inagglutinable).

Serum dilutions	1-80	1-160	1-320	1-640	1-1280	1-2560	1-5120
Agglut. of normal bac.	—	—	—	—	—	—	—

"B." Same serum not saturated.

Serum dilutions	1-80	1-160	1-320	1-640	1-1280	1-2560	1-5120
Agglut. of normal bac.	+	+	+	+	+	—	—

As the inagglutinable strain, therefore, absorbed the agglutinin from the homologous anti-serum, it was next resolved to test whether the absorptive power of the inagglutinable strain was greater or less than that of the normal strain.

Experiment 2. In this experiment the absorptive power both for homologous and heterologous agglutinins was tested: 1 c.c. of the immune serum was mixed with an equal bulk of the bacillary emulsion. The emulsion was prepared by adding 4 c.c. of normal saline to a 24 hours agar slope growth, and shaking till thoroughly broken up. The emulsions were standardised either by counting or by the opalescence method so that the number of bacteria present corresponded in each test as far as possible. The mixtures were kept for 2½ hours in the incubator, then centrifugalised, the clear fluid pipetted off and amount of agglutinin present estimated.

The anti-typhoid serum used had a titre of 1 in 5000 and the homologous serum a titre of 1 in 1250 for a normal strain of *B. typhosus*.

	Agglut. before absorption	After absorp- tion with normal typhoid	After absorption with inagglut. typhoid
Anti-typhoid serum (dil. 1 in 4)	1-5000	1-320	1-160
Anti-inagglut. serum ...	1-1250	1-160	1-80
Anti-typhoid serum (dil. 1 in 10)	—	1-600	1-320
Anti-inagglut. serum ...	—	1-320	1-160
Anti-typhoid serum (dil. 1 in 30)	—	1-880	1-220
Anti-inagglut. serum ...	—	1-440	1-220

The above experiments show that there is no loss of the absorptive power for agglutinin. There is indeed some evidence of increased absorptive power.

It was also found that the inagglutinable bacillus could absorb the group agglutinins for the *B. gaertner*, as the following experiment shows.

Anti-inagglutinable bacillus serum (after absorption by the inagglut. bacillus):

Serum dilutions	1-80	1-160	1-320	1-640	1-1280	1-2560
Agglut. of <i>B. gaertner</i>	—	—	—	—	—	—

Ditto (before absorption by the inagglut. bacillus):

Serum dilutions	1-80	1-160	1-320	1-640	1-1280	1-2560
Agglut. of <i>B. gaertner</i>	+	+	+	—	—	—

An attempt was then made to discover whether the converse was true, namely whether the inagglutinable bacillus after complete saturation with agglutinin behaved as the normal bacillus on digestion with saline.

Exp. Equal quantities of an anti-typhoid serum (1.0 c.c. of a 1 in 4 dilution) were placed in two centrifuge tubes; to one tube "A," 0.25 c.c. of an emulsion of the inagglutinable bacillus were added, and to the other, "B," a corresponding amount of the agglutinable bacillus. The tubes were then placed in the incubator at 37° C. for 2½ hours, at the end of which time they were centrifugalised and the fluid pipetted off. About 1.0 c.c. of saline was added to each, the tubes shaken and immediately centrifugalised and the saline pipetted off. In this way the greater part of the remaining anti-serum was removed. To each deposit of bacilli 1.0 c.c. of saline was added, the tubes well shaken and then allowed to digest at 37° C. for three hours. They were then centrifugalised and the agglutinating power of the fluid tested. Finally the deposit was digested with 1.0 c.c. of saline overnight and the agglutinating power of the supernatant liquid tested.

First digestion of saturated inagglut. bacillus. (A):

Dilutions	1-8	1-16	1-32	1-64	1-128	1-256
Agglut.	++++	++++	+++	++	0	0

First digestion of saturated agglut. bacillus. (B) :

Dilutions	1-8	1-16	1-32	1-64	1-128	1-256
Agglut.	++++	++++	++++	+++	+	0

Digestion over night.

(A)

Dilutions	1-8	1-16	1-32	1-64	1-128	1-256
Agglut.	++++	++++	++	0	0	0

(B)

Agglut.	+++	++	+	0	0	0
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In the above experiment both the inagglutinable and agglutinable bacillus behave with regard to the liberation of absorbed agglutinin in a similar manner, perhaps with the inagglutinable bacillus the fixation is not quite so firm, as in the digestion overnight slightly more agglutinin was liberated.

(2) *Aggregation.* From Bordet's and Gengou's researches it would appear that the determining factor in the aggregation of bacteria in agglutination is a change in the surface tension of the fluid in which they are suspended. The addition of certain substances which bring about agglutination (chemicals or specific sera) do so by modifying the molecular relations between the particles and the fluid.

More recently it has been suggested that all particles suspended in a liquid remain separate on account of a repellant electrical charge carried by the particles. Many bacteria and electro-negative colloids are precipitated by acids, that is, by the positively laden H-ions.

On the above hypothesis Michaelis (1911) and his pupil Beniasch (1912) showed that certain bacteria, especially those of the typhoid-colon group, were precipitated by definite H-ion concentrations, each having an optimum concentration of precipitation. This reaction they say is of a highly specific nature, and depends on the same phenomena as serum agglutination.

The following experiments show the behaviour of the inagglutinable strain of typhoid towards these chemical agglutinants.

Saffranin. Both the normal and the inagglutinable strains were agglutinated by saffranin in dilutions up to 1 in 3000.

Formalin. With formalin no difference was observed between the two strains. With a 50 % dilution no apparent agglutination beyond a slight formation of clumps was seen.

Acid agglutination. In applying the acid agglutination test concentrations of acetic and lactic acids were employed according to the formulae of Michaelis, Beniasch, and Heinmann (1913).

Exp. Acetic acid. A series of six concentrations of H-ions was made up according to Michaelis and their effects on the inagglutinable and normal bacilli tested. About 1 c.c. of each concentration was placed in a series of small test tubes and then 0.1 of the constant bacillary emulsion added to each.

Series:—	1	2	3	4	5	6
n. NaOH	5 c.c.	5 c.c.	5 c.c.	5 c.c.	5 c.c.	5 c.c.
n. CH ₃ COOH	7.5 c.c.	10 c.c.	15 c.c.	25 c.c.	45 c.c.	85 c.c.
Water dis.	87.5 c.c.	85 c.c.	80 c.c.	70 c.c.	50 c.c.	10 c.c.
H-ion concn.	1.0×10^{-5}	2×10^{-5}	4×10^{-5}	8×10^{-5}	1.6×10^{-4}	3.2×10^{-4}
<i>B. typhosus</i> inaggl.	0	0	++++	+++	+	0
<i>B. typhosus</i> lab.	0	+	++++	+++	++	0
<i>B. typhosus</i> Smith	0	+	++++	+++	++	0

++++ = complete sedimentation. The results were read after 2 hours at 37° C.

Exp. Lactic acid. The concentrations in this series were made up according to the formula of Heinmann, the technique being otherwise similar to that of the previous experiment.

Series:—	1	2	3	4	5	6	7	8
n/10 Sod. lactate	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.	0.5 c.c.
n/10 Lactic acid	0.12 c.c.	0.25 c.c.	0.5 c.c.	1.0 c.c.	—	—	—	—
n/1 Lactic acid	—	—	—	—	0.2 c.c.	0.4 c.c.	0.8 c.c.	1.6 c.c.
Water dist.	1.48 c.c.	1.35 c.c.	1.1 c.c.	0.6 c.c.	1.4 c.c.	1.2 c.c.	0.8 c.c.	0.0 c.c.
(H)	3.5×10^{-5}	7×10^{-5}	1.4×10^{-4}	2.8×10^{-4}	5.5×10^{-4}	1.1×10^{-3}	2.2×10^{-3}	4.4×10^{-3}
<i>B. typhosus</i> inagglut.	++	++++	++++	+++	0	0	0	0
<i>B. typhosus</i> lab.	++++	++++	+++	+++	?	0	0	0
<i>B. typhosus</i> Smith	++	+++	+++	++	0	0	0	0

In the above and preceding experiment no evidence of any sort was observed which indicated that the inagglutinable bacillus was more or less susceptible to precipitation by acids than the normal bacillus, the bacillus in question behaving like the control strains. The optimum concentration of H-ions which precipitate the inagglutinable bacillus is the same as that for the normal *B. typhosus*, namely 3.5×10^{-5} — 8×10^{-5} .

Complement fixation. Recent work has shown that by the use of suitable technique it is possible to distinguish between one member of the typhoid-colon group and another even though very closely related. This experiment was made in order that the antigenic power

of the inagglutinable bacillus in the fixation of the complement reaction might be compared with that of the normal bacillus.

Technique. The technique employed was based on that used in the Bacteriological Laboratory of the London Hospital, in performing the Wassermann reaction. The antigen was prepared by emulsifying a 24-hour agar slope growth in 4 c.c. of carbolic saline (0.5%). The necessary amount for the test was taken to be one-quarter of that amount which just gave complete inhibition of haemolysis after incubation for one hour at 37° C.; usually 0.02 c.c. In the haemolytic system a 5% suspension of sheep corpuscles was used and 3 units of amboceptor; of complement 2½ units were used. In the test the total volume was 1.5 c.c. of which 0.5 c.c. was haemolytic system.

Exp. With anti-typhoid serum:

Serum quantity	Inagglutinable strain	Agglutinable strain
0.05	++++	++++
0.025	++++	++++
0.02	++	++++
0.01	++	++++
0.006	+	+++
0.003	+	+++
0.001	0	+++
0.0005	0	++

With anti-inagglutinable typhoid serum:

Serum quantity	Inagglutinable strain	Agglutinable lab. strain
0.05	++++	++++
0.025	++++	++++
0.02	++++	++++
0.01	++++	++++
0.006	++++	++++
0.003	++++	+++
0.001	++++	+++
0.0005	+++	++

Argument. Inagglutinable strains or as they are sometimes called serum-fast strains of *B. typhosus* are undoubtedly of the same nature as the immune or fast races of protozoa. The foregoing experiments were made with the idea of finding some clue as to the mechanism by which such strains are able to resist the specific agglutin. There is reason to believe that the resistant strains arise from mere variants of the normal bacillus.

It has been suggested that the inagglutinable bacilli owe their peculiarity to a loss of their agglutinin receptors. Our absorption experiments however failed to demonstrate any loss or weakening of

the absorptive power for homologous or heterologous typhoid agglutinin. Müller, and Eisenberg and Volk found however with their strains some loss of absorptive power. There was some evidence in our experiments that the fixation of the agglutinin was not so firm, as it was more easily removed by washing than was the case with the normal bacillus.

Again complement fixation experiments failed to show any marked difference with regard to the antigenic power of the inagglutinable bacillus and the normal bacillus, though it seemed as if the inagglutinable bacillus was slightly more resistant to the heterologous than to the homologous amboceptor—a result frequently observed in complement fixation experiments.

It would therefore appear from the inoculation and other experiments that there is not a sufficient loss of agglutinin receptor or other receptors to account for practically a complete inagglutinability towards the specific agglutinin.

As regards the second part of agglutination—aggregation a purely physico-chemical phenomenon—no striking difference was found between the inagglutinable bacilli and the normal agglutinating strains of *B. typhosus*. They both behaved towards the chemical agglutinants in much the same way with the exception that occasionally the inagglutinable bacillus was slower in showing complete agglutination. The time limit in the acid agglutination experiments was $2\frac{1}{2}$ hours but the normal bacilli were often completely agglutinated within $1\frac{1}{2}$ hours while the inagglutinable bacillus only showed commencing aggregation. The physical agglutination is therefore apparently slightly delayed though there was no complete resistance as was the case with the specific anti-serum. Our experiments in this connexion do not support the view of Michaelis and his pupils, that acid agglutination and serum agglutination depend on the same factors.

Though our results are necessarily somewhat indefinite there is reason to believe that the phenomenon of inagglutinability is of a physical nature, the difference between the strains being of quality and not of kind.

CONCLUSIONS.

(1) By injections into rabbits, an inagglutinable strain of *B. typhosus* can produce specific agglutinin to the species *B. typhosus* though not to itself, except in the slightest degree.

(2) The injections also produce group-agglutinins for other members of the typhoid-colon group.

(3) The inagglutinable bacillus absorbs the agglutinin from both the homologous and heterologous antisera.

(4) The group agglutinins (for *B. gaertneri*) are also removed by absorption with the inagglutinable strain.

(5) There is little or no difference quantitatively between the absorptive power of the inagglutinable bacillus and the normal bacillus.

(6) The antigenic properties of the inagglutinable bacillus in the fixation of the complement reaction do not appear to be impaired to any extent.

(7) Chemical agglutinants, and acids in particular, act very similarly on both strains, though the reaction is slightly delayed in the case of the inagglutinable strain.

(8) The inagglutinable typhoid bacillus seems therefore to owe its peculiarity to some alteration of a physical character rather than to a loss of receptors which makes it more resistant to certain physico-chemical states which affect the normal bacillus in a certain definite manner.

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A STUDY OF SUMMER DIARRHOEA IN WARRINGTON IN 1911.

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IN the summer of 1911 in order to ascertain the sanitary conditions existing in houses in Warrington in which deaths from epidemic diarrhoea of patients under one year of age had taken place, I visited the houses in all cases where the entry on the death certificate issued by the medical practitioner was such as to suggest the possibility of the death having occurred from this disease. The total number of houses visited was 66.

The enquiries were framed with a view of eliciting the chief symptoms of the illness, the method of feeding, the nature, source, and conditions of storage of the food, the sanitary conditions of the home and the social status of the parents. Information only on the more striking results of the enquiry are given below.

A. Clinical aspects.

A following definition of epidemic or summer diarrhoea will probably receive wide acceptance—a disease of sudden onset in which the passage of frequent, much altered stools is almost invariably accompanied by a severe degree of vomiting and early collapse—the acute phase of the disease terminates within a week or ten days.

My enquiry made it evident that this type of diarrhoea is relatively very rare; the definition, however, describes the fulminating cases admitted into the wards of general hospitals. The rarity of this type of the disease possibly accounts to some extent for the difficulty experienced by the general practitioner when called upon to certify the form of disease of the alimentary tract from which a patient has died.

The questions asked of a clinical nature included the following:—

- (1) Was the illness of sudden onset?
- (2) Did the child vomit?
- (3) Did the vomiting continue up to the last?
- (4) Did the belly become larger?
- (5) Were the stools green; offensive; frothy; watery?
- (6) Did the stools contain slime, blood or curds?
- (7) Did the face of the child look pinched at the last?
- (8) Did the child have cramps in the stomach?

The history of the illness in 67 patients was investigated.

A sudden onset of the disease occurred in 38 patients, a gradual onset in 24; the parents of five patients could give no definite information on this point.

In the case of the patients whose illness commenced suddenly vomiting occurred in 29; the vomiting was of a severe degree in 14, a moderate degree in 6 and a slight degree in 9. No vomiting occurred in 7 cases; no definite information on the point was available in 2 cases.

Definite evidence in relation to vomiting was obtained in regard to 62; of these 46 (72%) vomited. It is worthy of remark that vomiting was stated to be present in 72% of cases investigated by Dr Niven in Manchester in 1905.

Slime was observed in the stools in 15 cases, and blood in 4; watery stools were noticed in 4 cases; watery and green stools in 3, and green but not watery in 26. In 18 cases the motions were very offensive. Eleven patients suffered from convulsions. In only one case with a sudden onset did severe vomiting and watery stools occur.

B. Multiple invasion of houses.

In Warrington instances of multiple invasion of houses occurred in connection with 19 (29 %) of 67 fatal cases investigated. By asking the mother whether any other person living in the house had suffered from diarrhoea either before or after the baby commenced to suffer from this complaint, some information was obtained as to the occurrence of this symptom and it was ascertained that the majority of these associated patients suffered from severe diarrhoea; it is probable that many cases of "looseness of the bowels" escaped the notice of the informant.

Persons suffering from this slight degree of abnormality may be suffering from the effects of a bacterial invasion of the alimentary tract; for this reason therefore an understatement may have been made of the number of cases in which the disease was acquired from another person in the house already suffering from diarrhoea.

The information obtained relating to multiple infection is given in tabular form below:

- No. 4. The father suffered from diarrhoea on a Monday and Tuesday; he was under the care of a doctor for 14 days; recovery took place.

The baby aet. 5 weeks commenced to suffer from diarrhoea on the subsequent Wednesday; the father looked after him for a short time; the illness of the baby lasted 17 days.

The sanitary conditions of the house were excellent.

- No. 7. Two other children had diarrhoea (3-4 motions a day) after the baby's illness had commenced.

- No. 9. One girl aet. $1\frac{3}{4}$ years and another aet. $2\frac{4}{5}$ years had diarrhoea before the infant.

- No. 13. A boy aet. $2\frac{6}{7}$ years had diarrhoea for 2 months subsequently to the onset of the disease in the baby whose illness lasted 2 months.

- No. 20. The grandmother living in the same house had diarrhoea for 2 months subsequently to the onset of diarrhoea in the baby.

- No. 25. The baby suffered from diarrhoea for 14 days; an interval of a few days elapsed before a second attack occurred.

A boy aet. 3 years had diarrhoea after the baby commenced to suffer from the second attack.

A girl aet. 2 years suffered from diarrhoea after the baby started with the second attack.

The diarrhoea in the case of these two children lasted for a day or two; they had not been eating fruit just before the attack.

- No. 26. A girl aet. 3 years had diarrhoea subsequently to the onset of the attack in the baby; she had been eating apples just before the attack.

- Nos. 29 and 30 (illegitimate twins). Mary II—died Aug. 28th; she was ill for 3 weeks; Muriel II—died Oct. 4th—the diarrhoea started on Sept. 23rd, i.e. 25 days after the death of her sister; she was ill for 11 days.

No. 37. A girl aet. 2 years suffered from diarrhoea on a Monday; it lasted for 15 days. The baby had diarrhoea on the subsequent Thursday; death took place.

Another child, a girl aet. 6 years, is in the Infirmary suffering from "windy bowels"; the disease commenced with diarrhoea.

No. 38. The baby commenced to suffer from diarrhoea on August 27th—he died on August 29th.

The father suffered from diarrhoea subsequently to the attack in the baby; later the mother and the children, aet. 2, 7, 9 and 10 years respectively were attacked: all the members of this household were attacked.

No. 41. The mother had diarrhoea which commenced 2 days after the onset of the disease in the baby.

No. 45. One of twins—the other had diarrhoea at the time of my visit a few days after the death of the first child—death did not occur in the case of the second child.

No. 47. A boy aet. $1\frac{1}{2}$ years had diarrhoea before the onset of the attack in the baby.

No. 48. The mother stated that all her children were "purged a bit." The first group affected comprise Aunie and Herbert who first suffered from diarrhoea on July 6th, 3 weeks before the baby started.

The father was off work suffering from "pilea" 3 weeks before the baby was taken ill.

The second group comprise Lily, Edie, and Elsie who commenced with diarrhoea "the last week but one before the baby," i.e. July 12th.

The third group comprise the baby whose illness started on July 27th. The mother while the baby was ill, i.e. from July 27th to August 10th, although not "purged" had colicky pains.

The fourth group comprise Samuel and Stanley who suffered from diarrhoea on August 10th and 11th.

No. 52. The baby's illness commenced on August 1st; he died on August 4th. A boy aet. 16 years suffered from diarrhoea which commenced on August 6th.

No. 55. A boy aet. 7 years had diarrhoea after the onset of the illness in the baby.

No. 60. The mother had diarrhoea which commenced two days after the death of the baby who had been ill for 17 days.

No. 65. The mother had "ptomaine poisoning" (with diarrhoea) for two days before the baby was taken ill with diarrhoea.

In the two following cases the proximity of a previous case of diarrhoea suggests the probability of this being the source of infection.

No. 33. Three of the family living next door had diarrhoea a few days before the onset of diarrhoea in this patient.

No. 47. A boy aet. 2 years living next door had diarrhoea a few days before the patient was taken ill.

Simultaneous onset in primary cases occurred in only two out of the 16 households, a simultaneous onset in secondary cases in three; this may be accounted for by simultaneous infection from the primary case.

Consecutive onset was the rule; only in one instance however were all the (seven) members of a family attacked more or less consecutively.

An average interval of eight days occurred between the onset in the primary and secondary cases. In only one instance did the secondary case succeed the primary case within four days; the usual interval

varied between four and nine days; longer intervals of which three weeks was the exception.

An instance suggesting how diarrhoea spreads from one house to another is seen in the case of No. 21. This child died of diarrhoea at No. X, *a* Street on September 14th. Her uncle, a baby (No. 11) had died of diarrhoea at No. Y, *β* Street on August 13th. The mother of the patient No. 11 spent much of her time at No. X, *a* Street and helped her mother to attend to the baby while he was ill.

Observations by Drs Niven, Sandilands, Peters and Dudfield have previously shown the frequency of multiple attacks in houses. Dr Niven in 1904 found that multiple attacks had occurred in 36 families in connection with 111 fatal cases (32% of the invaded families); in 35 fatal cases investigated by Dr Sandilands, 12 families (34%) showed multiple invasions; in Dr Peters' series in 83 (47%) out of 174 invaded families multiple cases occurred.

Dr Dudfield's observations showed that 41 families with multiple cases were found in connection with 423 cases (9·8%).

C. Consecutive date of onset in multiple cases.

It might be argued that even if invasion of houses or families occurs, the disease might be due to the action of a common cause such as the consumption by the several members of the household of contaminated milk. The consecutive date of onset in such cases, however, suggests that the disease was communicated from one person to another.

As regards the Warrington series in connection with this point, the collection of cases of which No. 48 forms one exhibits in marked degree the successive onset of the disease; in this set all the members of the household were affected; the cases occurred in four groups. In connection with No. 38 all the members of the household suffered from diarrhoea and although the cases cannot be absolutely differentiated into several groups, three groups could be distinguished.

In the following cases where particulars on the point have been ascertained, details are given as to the interval between primary and subsequent cases:

In connection with patient No. 4.	2 days intervened between the onset of the primary case (which lasted 14 days) and the secondary.
.. .. patient No. 37.	3 days intervened (the primary case lasted 15 days).
.. .. patient No. 41.	2 days intervened (the secondary case commenced 2 days after the onset of the illness in the primary case).

In connection with patient No. 48.	6 days intervened between the primary and secondary cases. 15 days intervened between the secondary and tertiary cases. 12 days intervened between the tertiary and quaternary cases.
.. .. patient No. 52.	5 days intervened between the primary and secondary cases.
.. .. patient No. 60.	19 days intervened between the primary and secondary cases (the primary case was ill 17 days).
.. .. patient No. 65.	2 days intervened between the primary and secondary cases.

An average of 5.5 days therefore intervened between the primary and secondary cases.

The phenomenon of simultaneous onset in secondary and subsequent groups of cases occurred in connection with patients Nos. 38 and 48.

In the course of an investigation into deaths from diarrhoea which occurred in Kensington in the third and fourth quarters of the year 1909, Dr Sandilands obtained records of twelve families in which multiple cases of diarrhoea had occurred.

Simultaneous onset in primary cases occurred in only two of the 16 households; a simultaneous onset in secondary cases in three; this, however, may be accounted for by simultaneous infection from the primary case. Consecutive onset was the rule; only in one instance were all the (seven) members of a family attacked more or less consecutively.

An average interval of eight days occurred between the primary and secondary cases; "in only one instance did the secondary case succeed the primary case within four days; the usual interval varied between four and nine days; longer intervals of which three weeks was the maximum period recorded, were bridged by continuous illness in the primary case."

Dr Dudfield in Paddington was unable to demonstrate the consecutive dates of onset in the instances of 32 patients (who belonged to eight houses) suffering from diarrhoea; only two of these patients fell ill on the same day.

D. *The diarrhoeal death rate in the several wards of a town.*

When the prevalence of diarrhoea is receiving consideration much attention is devoted to the meteorological conditions and especially to the temperature either of the earth or atmosphere and the rainfall. For all practical purposes however we are justified in assuming that the temperature and rainfall in the several wards of a town with a population of 70,000 are identical.

The following table is a comparison of the average diarrhoeal death rate for the several wards of Warrington with the average birth rate, the zymotic rate corrected for zymotic diarrhoea, and the relative poverty or otherwise of the inhabitants of the ward.

TABLE I. *Comparison of average diarrhoeal death rate, birth rate, corrected zymotic rate and rateable value of dwelling houses.*

Ward	No. of deaths from diarrhoea	Average diarrhoeal death rate	Position in list	Average birth rate	Position in list	Average zymotic rate corrected for diarrhoea	Position in list	Average rateable value of dwelling houses	Position in list
St Austin's	99	·1071	1st	22·3	1st	1·8933	1st	£16·34	2nd
Town Hall	146	·148	2nd	25·3	2nd	2·224	2nd	£16·77	1st
Latchford	197	·1603	3rd	30·0	3rd	2·240	3rd	£11·71	4th
Bewsey	184	·2029	4th	31·1	4th	3·8176	8th	£11·18	6th
White Cross	247	·2045	5th	39·0	9th	3·23	5th	£10·17	8th
Fairfield	222	·2072	6th	32·0	5th	2·891	4th	£11·33	5th
Howley	255	·2194	7th	36·8	7th	3·4189	6th	£11·97	3rd
St John's	412	·2475	8th	37·7	8th	4·097	9th	£9·21	9th
Orford	309	·2653	9th	33·1	6th	3·711	7th	£10·19	7th

The average diarrhoeal death rate for each ward has been calculated from the rates for the years 1892-1910 inclusive; the average birth rate has been calculated for the nine years previous to and including 1910. It is only claimed that the position in the list in each case is comparable.

A close relation is seen to exist between the birth rate and the diarrhoeal death rate; the higher the birth rate in a ward the higher is the mortality from diarrhoea.

A deviation from this rule is seen in the case of White Cross Ward which has the fifth lowest diarrhoeal rate but the highest birth rate. The vital statistics of this ward have been, as a rule, comparatively satisfactory, *e.g.* in 1903 the Medical Officer of Health commented on the high birth rate (40·2) compared with the birth rate (35·6) for the whole of the town and the comparatively low general death rate (14·3 for the ward, 18·4 for the whole of the town) and infantile mortality rate (125 for the ward, 154 for the whole of the town). Marked features of this ward are the modern construction of the houses and the absence of overcrowding of the area with houses.

The correspondence in position in the table of the diarrhoeal rate and the zymotic rate (corrected for the diarrhoeal rate) is very close with the exception of Bewsey Ward. This ward while relatively free

from diarrhoea has suffered extensively from zymotic disease; no obvious explanation, however, presents itself.

The general close correspondence between these two rates suggests that diarrhoea is of the nature of an infectious disease.

A comparison of the incidence of typhoid fever and the mortality rate from diarrhoea in the several wards of the town is given in the following table :—

TABLE II. *Incidence of typhoid fever and mortality from diarrhoea in the several wards of Warrington.*

Ward	Average diarrhoeal rate	Position in list	No. of cases of typhoid fever 1892—1910	Average population 1892—1910	Average rate of incidence	Position in list
St Austin's	·1071	1st	37	5163	·716 ÷ 19	1st
Town Hall	·148	2nd	57	5536	1·029 ÷ 19	4th
Latchford	·1603	3rd	65	8223	·79 ÷ 19	2nd
Bewsey	·2029	4th	57	5430	1·049 ÷ 19	5th
White Cross	·2045	5th	119	8139	1·46 ÷ 19	7th
Fairfield	·2072	6th	63	7097	·88 ÷ 19	3rd
Howley	·2194	7th	102	6073	1·529 ÷ 19	8th
St John's	·2475	8th	198	9892	2·001 ÷ 19	9th
Orford	·2653	9th	104	8113	1·28 ÷ 19	6th

While a certain amount of correspondence of position in the list appears to exist, the degree of correspondence is much less than in the other data compared, and on the whole the evidence tends to show that there has been no close correspondence between the incidence of typhoid fever and the deaths from diarrhoea in the several wards of the town.

This is also borne out by a special Report by the Medical Officer of Health in 1899 which attempted with considerable success to connect the prevalence of typhoid fever with a particular part of the town; diarrhoea was fairly evenly distributed, showing no preponderance in one district over another.

The general opinion of epidemiologists is that diarrhoea is a class disease and that it affects the poorer classes of the community, the middle and upper classes being comparatively unattacked by the disease.

A measure of the relative poverty or otherwise of the inhabitants of a ward is afforded by the average gross rateable value of each dwelling house in the ward. In ascertaining these figures for the wards of Warrington care has been taken to eliminate from the calculation those buildings such as the public buildings, workhouse, hospitals, barracks

and shops, the inclusion of which, for obvious reasons, would render the comparison less accurate.

On reference to Table I a general correspondence between a low diarrhoeal rate and a high average gross rateable value is seen to exist—the most striking exception is seen in the case of Howley Ward. The outstanding particular in which this ward differs from the rest of the town is its low-lying situation. In Woolwich it was found that, as a rule, the highest diarrhoeal incidence and death rate occurred in districts situated on the lowest levels and vice versâ. Dr Davies goes on to say:—"This may be partially due to the poorer class of population being usually found on the lower ground but the whole of the difference could hardly be thus explained."

E. *Sanitary conditions inside houses.*

(1) *Overcrowding.* In my series of cases the total number of occupants of 324 rooms (66 houses) was 405, an average of 1.25 persons per room; the average number per house was 6.13.

The mean population of Warrington for the year 1911 was 72,375; assuming that the number of inhabitants of the Poor Law institutions, barracks and hospitals was 600 (an under-estimation rather than an over-estimation), the number of persons occupying 14,124 houses (the number of occupied houses in June, 1911) was 71,675 or 5.07 per house. Relative overcrowding, therefore, occurred in those houses in which deaths took place from diarrhoea.

In the Woolwich series of cases, in 99 out of 820 houses there were more than two persons to a room, *i.e.* in about 12% there was overcrowding. The estimated proportion of overcrowding in the Borough is decidedly under 1%, "so that there is reason to think that overcrowding greatly affects the prevalence of diarrhoea."

The degree of overcrowding in both series of cases is shown in the following table:

TABLE III. *Overcrowding and diarrhoea.*

	Total No. of cases	No. under 1.5 persons to a room	No. with 1.5 and under 2 persons to a room	No. with 2 or more persons to a room
Woolwich	820	516 (62.9%)	204 (24.9%)	100 (12.2%)
Warrington	67	45 (67.1%)	13 (19.4%)	9 (13.4%)

Dr Dudfield, however, as the result of his enquiry in Paddington in 1911, considers that the number of occupants per house has very little, if any, influence on the prevalence of diarrhoea.

(2) *Cleanliness of houses.* In 48 of my series of cases, the house was clean, in 17 fair and in 2 dirty. Eleven of the children were entirely breast fed; in these eleven cases 5 houses were clean, 4 fair and 2 dirty; in the other 56 cases 43 houses were clean, 14 fair and none dirty; these figures indicate that the houses were less clean in the case of the entirely breast fed children who died of diarrhoea than in the other cases and points to a possible reason why, in spite of their having the advantage of being breast fed, they contracted the disease.

A comparison of the sanitary conditions in the houses of the children who were healthy and those who were unhealthy before the fatal attack of diarrhoea shows that the houses were somewhat more cleanly in the case of the healthy children. The figures are given below:

TABLE IV. *Cleanliness of houses of children who have died of diarrhoea.*

Cleanliness of home	Child healthy before attack	Child unhealthy before attack
Satisfactory	30 (75 %)	18 (69 %)
Medium	10	7
Unsatisfactory	1	1

F. *Sanitary conditions outside houses.*

(1) *Ashpits.* The condition of the ashpits belonging to the houses visited in Warrington was unsatisfactory, although in almost every case the ashbin was of the galvanised iron variety and not the old-fashioned receptacle made of brick with a wooden cover. The top of the bin was found in position in one case only out of 66 houses visited.

There were various degrees of defect existing but the net result in the majority of cases was the existence of a potential offensive surface more or less exposed to the air of the back yard and serving both as a medium for bacterial growth and a breeding ground for flies in the neighbourhood of the houses.

(2) *Proximity to stables &c.* In 22 instances stables were in the proximity of the houses in which deaths from diarrhoea took place. In two of these instances two stables were near the house; in another case a tannery in addition to a stable was near the house. In two other instances a nuisance of a similar nature was close to the house; in one case a tanyard, in the other a middenstead.

The following table (V) gives a comparison of the number of flies present in the household, near stables, &c. and the other households:—

TABLE V. *Number of flies present in houses of children during a fatal attack of diarrhoea.*

	Households near stables, &c.	Other households
Many flies seen in the house during illness	9 (37.5 %)	12 (36.3 %)
Moderate number of flies seen in the house during illness	9 (37.5 %)	1 (3.3 %)
Few flies only seen in house during illness	6 (25 %)	20 (60.6 %)

The table shows that the number of flies were more abundant in the houses near stables than in the others.

I have to thank Dr J. Coote Hibbert, now Medical Officer of Health of Blackburn, and Dr Graham-Smith for their kind assistance.

SUMMARY AND CONCLUSIONS.

(1) The type of summer diarrhoea usually seen in the wards of general hospitals was rare in Warrington in the summer of 1911.

(2) Multiple invasion of houses occurred in 19 (29 %) of 67 fatal cases investigated.

(3) Consecutive onset of the disease among the members of the household was the rule.

(4) The average diarrhoeal rate for the several wards of the town varied directly as

- (a) the average birth rate;
- (b) the zymotic rate corrected for zymotic diarrhoea;
- (c) the relative poverty of the inhabitants of the ward.

(5) Relative overcrowding existed in the houses in which deaths took place from diarrhoea.

(6) Cleanliness of houses is an important factor in the prevention of diarrhoea.

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NOTE ON THE PLATE METHOD FOR ENUMERATION OF BACTERIA.

By ANDREW CUNNINGHAM, B.Sc. (Edin.).

IN recent years much work has been done on the bacterial content of milk, chiefly with a view to demonstrating the principal sources of contamination and at the same time collecting data to aid in the fixing of a bacterial standard for "clean" milk. On looking through the various publications one is particularly impressed by the lack of uniformity in the length of the period of incubation of gelatine and agar plates. Some observers adopt a period of only a few days while others prefer to incubate for 8-10 days before counting their plates. It does not yet seem to be generally recognised, in Britain at all events, that, if the highest figures possible are to be obtained, the incubation period must be longer than three days.

Further in such publications, especially where the longer period has been adopted, one too frequently finds that in a series of comparative tests one or more of the gelatine plates has become entirely liquefied and uncountable before the end of the incubation period has been reached, and in this way the whole series has been more or less spoiled. Such upsetting results could, however, be entirely avoided by the simple method, suggested by Hiltner and Störmer¹, of touching the liquefying colonies while still young with the fine point of a stick of silver nitrate, when, if the medium contains a chloride, a white precipitate of silver chloride forms round each colony and prevents further development. The chief difficulty in the carrying out of this suggestion lies in the recognition of the liquefying colonies while they are still so young that they have not excreted any appreciable amount of the liquefying enzyme. Still, with a little practice this difficulty can be very easily surmounted.

¹ *Arb. u. d. biol. Abt. d. Kais. Gesundheits.-Amts.* III. p. 449, 1903.

The work discussed in this note was undertaken with a view to:

(1) Demonstrating the length of time required for the incubation of gelatine and agar plates in order to secure the maximum development of colonies.

(2) Showing the advantages of treating liquefying colonies on gelatine plates with silver nitrate in order to retard development.

(3) Incidentally the numbers of bacteria developing on agar at 22° C. and at 37° C. and on gelatine plates have been compared.

The materials employed for the enumerations were soil, dung and milk and ordinary 12% gelatine and 1.5% agar, made just alkaline to litmus, were used as media. Dilutions were made in the usual way and the plates showing a convenient number of colonies for counting were selected. As the results are merely intended for demonstration purposes and do not bring out anything new, it was not thought advisable to accumulate a great number of data.

Table I shows the length of time required for the number of colonies on the various plates to reach a maximum¹.

In the case of the gelatine plates it will be seen that the maximum for soil and dung is reached in about five days; for the milk sample, however, about nine days elapsed before the maximum was attained.

TABLE I.

No. of days	Gelatine			Agar at 22° C.			Agar at 37° C.		
	Soil	Dung	Milk	Soil	Dung	Milk	Soil	Dung	Milk
1	—	—	—	—	—	—	—	—	—
2	20	48	14	11	7	24	29	20	171
3	57	104	44	17	13	59	36	23	186
4	72	115	49	34	20	88	36	23	—
5	76	117	50	43	26	95	36	24	—
6	76	117	50	55	32	97	36	24	—
7	76	117	51	59	39	100	Plates dry		
8	78	118	55	60	44	100	—	—	—
9	78	118	61	61	45	100	—	—	—
10	79	118	62	61	50	101	—	—	—

On agar at 37° C. the maximum was reached in each case in three days. On the third day the plate made from the milk sample was placed in the 22° C. incubator for seven days. This resulted in an increase from 186 to 222 or about 20%.

¹ The dilution employed varies, so that the figures are not comparable for the bacterial content of the materials. This is dealt with later.

On agar at 22° C. a maximum was not attained till the 8th to 10th day, and the dung plate required to be incubated two days longer, during which time it increased from 50 to 57. After ten days the milk plate was incubated further at 37° C. but no increase in the number of colonies took place.

It is specially desired to emphasise the importance of 8-10 days' incubation for agar plates at 22° C. as this appears to be a point which is frequently overlooked. Doubtless, when comparative figures only are required, three days at 22° C. may suffice but in all other cases it is absolutely essential to incubate for at least eight days. 10 days' incubation as compared with three has given an increase varying from about one to three times. For gelatine plates 8-10 days' incubation is required and for agar at 37° C., three days.

In Table II a comparison is made between the number of colonies developing on ordinary gelatine plates and the number obtained on gelatine plates on which the liquefying colonies have been treated with silver nitrate while still small.

TABLE II.

No. of days	Soil		Dung		Milk	
	Gelatine untreated	Gelatine with silver nitrate	Gelatine untreated	Gelatine with silver nitrate	Gelatine untreated	Gelatine with silver nitrate
1	—	—	—	—	—	—
2	21	20	46	48	18	14
3	Plate liquefied	57	96	104	52	44
4		72	120	115	54	49
5		76	136	117	59	50
6		76	Plate liquefied	117	61	50
7		76		117	63	51
8		78		118	Plate liquefied	55
9		78		118		61
10		79		118		62

The advantages of this method of treatment are most obvious in the cases of soil and milk, in both of which the ordinary plate was liquefied before the other had attained its maximum. This is particularly marked in the case of soil, which, however, was only to be expected as here one is dealing with a medium containing large numbers of liquefying moulds. In the enumeration of bacteria in soil, therefore, the method of treating liquefying colonies with silver nitrate is specially to be recommended.

It should be observed that in practically every case the plate treated with silver nitrate has shown a slightly smaller number of

colonies than the untreated plate, *i.e.* comparing the numbers on the same day. This may possibly be due to the effect of the necessarily considerable quantity of silver chloride on the treated plate. Still one must observe that on the average a higher number will be found on the treated plate at the end of incubation than on the untreated before liquefaction.

Table III gives the numbers of bacteria per gram or cubic centimetre of the material as indicated by the growth of colonies on gelatine and agar plates.

TABLE III.

	Soil (gram)	Dung (gram)	Milk (c.c.)
Gelatine	7,900,000	1,180,000,000	6,200,000
Agar at 22° C.	6,100,000	570,000,000	1,010,000
Agar at 37° C.	360,000	2,400,000	222,000

It is particularly noteworthy that in each case the highest numbers are obtained from gelatine plates; next comes agar at 22° C. and lastly agar at 37° C. These results are very much as one might expect from a consideration of the sources of the bacteria in each material. In soil in temperate climates the organisms are accustomed to low temperatures and so might be expected to grow well at 22° C. Thus the two plates (gelatine and agar) incubated at 22° C. gave very nearly the same result, while that kept at 37° C. gave a very much lower figure. In the case of dung, if the material is fresh (as was the case in the sample examined), the same remarks apply: but in an old rotting sample the bacteria which thrive best at 22° C. do not flourish and are replaced by the multiplication of others which are better adapted to the high temperature prevailing in the material. In an ordinary sample of market milk one might also expect the greatest development at 22° C., since most of the bacteria in such samples are derived from the air and from the skin of the cow and are thus accustomed to temperatures in the neighbourhood of 22° C. This view is supported by the fact that the 37° C. agar plate on incubation at 22° C. showed a further increase, whereas the numbers on the 22° C. agar plate remained stationary when the latter was incubated at 37° C. (see above).

Further, dung contains considerable quantities of easily decomposable nitrogenous material and, therefore, one may presume, bacteria specially suited to a highly nitrogenous medium. This may partially account for the optimum growth of bacteria on gelatine. In the case of soil, although the content of nitrogenous organic material is not great, we

know from ammonification and other experiments that large numbers of organisms are present which might be expected to flourish on a nitrogenous medium.

Considering that gelatine appears to be such an excellent medium for the growth of bacteria from soil, dung and milk there would seem to be no adequate reason why it should not be more frequently employed especially as the introduction of the silver nitrate method for the killing of liquefying colonies has made it practically as permanent as agar.

In conclusion, I wish to thank Prof. Löhnis for having suggested this piece of work and also for advice and suggestion during the carrying out of the same.

THE EFFECTS OF NITROGEN-PEROXIDE ON THE CONSTITUENTS OF FLOUR IN RELATION TO THE COMMERCIAL PRACTICE OF BLEACHING FLOUR WITH THAT REAGENT.

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THE process of subjecting flour to the whitening action of dilute nitrous fumes has grown so extensively during the past ten or twelve years, that it is estimated that more than half of the enormous amount of flour utilised for making bread and for other purposes is now bleached or tinted by this method. Yet none of this bleached or artificially whitened flour is sold to the public as such specifically, or marked by any distinctive label, to show that it has passed through a chemical process.

This is an extraordinary condition of affairs, and as the matter concerns one of the most important of all our foodstuffs, and indeed that one which especially forms a preponderating constituent in the daily diet of the teeming millions of poorer inhabitants in the country, the urgency of a national consideration of the whole situation becomes obvious.

It is about time that the question was asked, "Why is flour bleached in these enormous quantities and who benefits by the process, the miller,

¹ The experiments recorded in this paper were carried out in collaboration at the Johnston Bio-chemical laboratory of the University of Liverpool and at the laboratories of the County Council of Lanark, and were part of the evidence in the complaint heard in 1912 by Sheriff Shennan of Dr Wilson against Uddingston Co-operative Society, in which judgment was given for the defendants.

the baker, or the consumer, or do they all share in the benefits of bleaching?"

The question has very broad economic and public health aspects, when it is remembered to what a large extent infants over six months of age and our vast population of school-children rapidly growing and requiring the most nutritious and wholesome food that can be given to them, are brought up on bread and food prepared in various ways from flour.

The money spent annually by millers in setting up bleaching plant and sending flour through the bleaching process if spent instead upon the solution and applications of health problems would probably suffice to provide all the school clinics, open-air schools and medical treatment required by our growing population. Even if it cannot be proven clearly in our present state of knowledge that bleaching, as commercially practised, is directly injurious to the chief constituents of flour, or exercises a slow chronic injurious effect upon the young and growing animal organism, still if no positively beneficial effect of the bleaching agent upon the flour can be demonstrated which makes it a better food-stuff, then the large sum spent on bleaching can only be set down as a national waste which ought to be prevented on the grounds of national economy, if for no other reason.

Now, the supporters of the process of flour bleaching have not been able to produce one shred of evidence that bleaching renders flour a healthier or more nutritious foodstuff than it would be in its natural unbleached condition. The most that can be claimed here is that there is a popular demand for a white bread which better suits some aesthetic taste, so that bleaching enables the miller to produce for the baker a white flour yielding a white loaf which pleases the eye and secures a preferential market for the product. A uniformity of colour at different times of year is also said to be established, and power of better utilizing and finding a market for the flour derived from darker-coloured foreign wheats. These things do not however mean any intrinsic improvement in the nature of the foodstuff and by so raising more highly coloured flours to the level of naturally white first-class flour a manipulation of the market is set in operation, causing the appearance of more fictitiously white high-class flours, and, for this whiteness, artificially produced, millers, bakers and consumers amongst them must pay. It is a curious fact that the supporters of bleaching claim that bleaching does not enable the miller to obtain a better price for the bleached product, but only secures for him a better and a more uniform

market. A little study of economics would soon teach those who advance such an argument that these two effects in the end amount to the same thing. A miller who cannot obtain a market must lower his prices, and if the lowering of his prices will not command a market, then the introduction of bleaching has gone still further than raising prices on behalf of those who do bleach, namely, it has spoiled the market for those who still persist in avoiding bleaching and wish to provide flour in its natural condition.

A miller is a business man, he is offered a process which will cost him roughly, say, one shilling per sack of flour to apply; if he takes up that process he will naturally expect to benefit by it to the extent, say, of one shilling and sixpence per sack in the flour he turns out. In the long run it matters little whether he uses his advantage to raise his price or to oust a rival from a market. When the market has adjusted itself, as it must do in a year or two, the cost of the bleaching must be found from somewhere, and either the millers and bakers are making less collectively, or the public is paying more for its bread, which means that the children are probably getting less. There is no escape from this economic position, and in the adjustment there is little doubt that a little of the imposed cost of bleaching, just like a tax, is borne by everybody along the line, from the sower of the wheat to the eater of the bread.

Before leaving the purely economic aspect of the question and passing to the action of the bleaching reagent on the flour, it may be of some interest to enquire briefly into the reason for this public fancy for white bread which is catered for by bleaching so as to give it artificially. An enquiry into its history will demonstrate two things: first, that the taste for the white bread had a reasonable and natural basis in the old purely mechanical process of milling before the introduction of chemical bleachers, and secondly that there is an intrinsic difference between first-quality white unbleached flour and the yellower second-quality flour now caused by bleaching it to simulate the former. This subtle difference is shown by the location of the oil. The difference cannot be clearly demonstrated by chemical examination of the constituents of the two qualities of flour, but microscopic examination demonstrates that the colour is an outward and visible sign of lack of ripeness in the endosperm, the oil and colour adhering to the younger, less completely developed granules, and it is for this reason that in the sifting or bolting process the whiter flour first separates at what is known as the head of the mill, and the yellower flour at the lower machines.

The wheat in the process of milling is passed through various sets of rollers of different grades, where in the earlier crushings the coarser offal is separated and the interior of the grain, or endosperm, is broken into coarse particles called semolina. In the later rollings the semolina is "reduced" or broken down by degrees into very fine powder and there are also present with it very fine microscopic particles of seed-hairs, epicarp and seed-coat.

These finely ground or rolled mixtures are passed after each rolling through a fine silk sieve or bolting, the portion which passes through being kept as a "stock" while that not passing through is sent on to the next machine. In this way a considerable number of "stock" fractions arise from the various machines and their attached sifters, which are numbered 1, 2, 3 etc. up to 12 or more, or in other cases as *A, B, C, D*, to *J*, or even *M*.

It may be mentioned that at the earlier stages of comminution of the grain it is not desired to crush the wheat grain completely. If this were done it would be difficult or impossible to separate the coarse offal from the fine part, and much of the valuable endosperm would be lost by it adhering to the offal. The first rollers are so designed and spaced that they only crack or *break* the grain and so yield coarse offal, as well as large or coarse particles of the flour-forming material.

In these first processes of breaking, however, small quantities of fine powder are unavoidably formed. These constitute the so-called "break" flours, and at various stages in comminution three or four "break" flours so arise in addition to the "stocks" mentioned above. These are poor products containing a good deal of microscopic offal, and a lesser amount of visible offal and unripe endosperm.

It has been necessary to give this brief account of the process of milling, because an understanding of the economics of bleaching turns upon it, and also the real differences between first and second grade flours are shown much more readily by following this process carefully than by any minute chemical examination of the various products. It also will become discernible, presently, what exactly bleaching has done for millers, bakers and the public.

When the products of the various sifting machines *A* to *J* are examined by the eye alone, it is seen that these become progressively yellower in colour, or "darker" as the miller usually calls it. The top machines give the highest commercial quality in colour and although they are the whitest and most devoid of colour, the miller calls it the "highest" colour, which has led to a good deal of confusion, and to

bleaching being called "tinting" the flour, although in reality it is the removal of tint.

The art of the miller before the introduction of "tinting" or "bleaching" consisted in skilfully blending the products of these various siftings. He made what was called a "divide" and the "top of the mill" or products *B* to *E* or *F* formed the finest, superfine or "patents" flour, and portions of these mixed with lower siftings formed "households" or "machine grades." The latter were of lower price, and were usually regarded as of lower quality. They might undoubtedly be quite nutritious, but were of coarser quality, just as pease-meal is most nutritious but less appetising than flour, cheaper to produce, and sells on the market for a lower price.

Now bleaching by removing the visible index given by the colour allows the miller to mix more of the product of the lower sifting machines with the upper and so obtain what is technically known as a "longer divide."

That this is so is shown by the advertisements of the "Flour Oxidising Co., Ltd.," who sell "tinting" or "bleaching" plant to millers, and as an inducement to purchase state *inter alia* in their advertisement²:—"The Corona³ gives you a better flour divide." "The Corona lifts the colour of your residue."

There is also evidence given by microscopical examination which clearly shows that lower "stocks" or fractions are in commercial practice so lifted or raised in colour that they can be included in the higher or "superfine" portion of the divide.

But before passing to this the question may be discussed—Is there any difference whatever save colour between the "stocks" or siftings, *B*, *C*, *D*, and *E* to *J*, which formerly made the first regarded as "patents" and the second as "households," and was it solely due to a difference in colour that one was commercially more valuable than the other? An examination will we believe show that there were, and are, other differences, and that the artificial removal of the colour by the process of "tinting" or "bleaching" has taken away a valuable guide which assisted the baker, or consumer, in purchasing a high-grade flour.

First, it may be asked, why do the top machines separate from the

¹ The product of the first machine *A* after the "Break" machines is not so good as *B*, *C*, *D*, which are regarded by millers as yielding the "cream of the wheat." Since this difference depends on other matters than colour and does not concern flour bleaching, the consideration of this point need not be entered on.

² *Milling*, Jan. 13th, 1912.

³ The name of the plant.

crushed grain a white product, and the lower machines a yellow product? The meshes of these silk sieves have no eyes to see colour and so would allow to pass indiscriminately a white or yellow powder. It is not the whiteness or yellowness that separates the fractions *A* to *J* as the grain is rolled down to flour, but the colour follows some difference of a physical character in the component parts of the mixed product from the crushed wheat. These component parts must differ physically in size or form or other property, which gives them greater or lesser facility for passing through the sieve. The colour properties are correlated to these properties which the sieve picks out, and so the colour is only a visible indicator of other differences between the first and second qualities. Removal of the colour will therefore disguise these other differences and render them invisible, but the separation in the sifting processes show they exist. Why else should there be a separation? It is absurd to claim that the products of the upper and lower machines differ only in colour, for that alone could not give passage to one particle through a sieve and refuse it to another.

A careful microscopic examination of the products of the different machines *A* to *J* discloses the cause of the separation and the intrinsic differences between first- and second-quality flours.

A minute quantity of each fraction or "stock" of flour is taken, mixed with a drop of water, and examined under the microscope. A cursory examination shows quantitatively the same main constituents in each case, but the *qualitative* distribution of the constituents differs progressively as the mill products are followed down, and a careful examination reveals that the ripest and best products of the endosperm are contained in *A* to *D*, and that the unripe, only partially developed, portions of the wheat appear in increasing quantity from *E* onward. "Tinting," by removing the colour index, allows the unripe to be mixed with the ripe and fully developed particles. In addition, while vegetable hairs and microscopic fragments of epicarp are practically absent from *A*, *B*, *C* and *D*, these commence to appear about *E* and are found in increasing quantity as the mill is descended from *E* to *J* or beyond. These, unlike the colour, are not removed by bleaching and form a valuable index as to whether by the aid of "tinting" first and second qualities have been mixed for the market.

The microscopic examination thus reveals in an interesting way why the various products have separated in the process of sifting and amply confirms the reasoning above that it is not colour that causes them to separate, but other intrinsic differences.

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The chief objects seen when mixed flour, as sold, is examined under the microscope, are large numbers of ripe fully-formed starch granules and smaller numbers of incompletely developed starch granules. Both of these are separate from anything else as round particles like microscopic pebbles; then there are agglomerations of tissue from the wheat cells containing minute granules of starch and oil. The masses containing these minute granules are much larger than the individual ripe starch granules existing separately. They vary enormously in size and shape and are irregular in form. They have the appearance of cell debris crushed out of shape and adherent. It is quite obvious on looking at them that these masses would have more difficulty as a rule, especially when they are larger, in getting through the mesh of a sieve.

Now, on making a microscopic examination separately of the various "stocks" or fractions from *A* to *J*, while these constituents are plentiful in all the fractions, there is a greater preponderance of the ripe starch granule in *A* to *D*, while more, comparatively, of the cell debris, with its unripe granules embedded in it, is found in *E* to *J*.

The *colouring matter* of the flour is contained in the small amount of oil present in the cellular matter, and hence the oil and the colour distribution follow the unripe material. Ripe starch granules predominate in the stocks from the higher machines which are thus poorer in oil and accordingly less coloured. It is therefore easy to see why the ripe starch granules predominate accompanied by lighter colour in the part which passes more readily through the bolting sieves, and why the cellular debris and less ripe part accompanied by the colour predominate in lower machines.

In addition to these differences in the relative distribution of the chief constituents of the flour, it is also observable that seed-hairs, small fragments of epicarp and of seed-coats, on account of their form, tend to be retained by the sieves, and passed on to the next machines, so that while the fractions of *A*, *B*, *C*, and *D* of the top of the mill are practically free from them, they occur in increasing abundance in the lower fractions *E* to *J*.

These microscopic particles are not changed by the bleaching process and hence may be utilized to determine how a given flour has been blended, in spite of any bleaching, or "tinting," which may have been employed either upon the lower fractions or the blended flour as a whole. In this way also a really top-grade flour may be distinguished from a lower grade bleached product.

At our request Dr J. Hume Patterson¹ made a microscopical examination of the "stocks" from the separate machines of the mill which are blended to form commercial flours. Dr Patterson also devised a method for counting the numbers of hairs, epicarp and seed-coat particles in these, and in various commercial flours, based on the same principle as that used by the physiologist for counting blood corpuscles, and by the bacteriologist for counting bacteria in a culture.

His results, which are reproduced in the accompanying tables, clearly show how these particles increase in numbers in the lower grade machines, and also the interesting and important fact that artificial "tinting" can be utilized to blend lower with higher grade flours, and so "give a better divide" as the Corona advertisement has it. It is certainly a better divide commercially for the miller, whether it is also a better divide for the baker and consumer is another question.

The method of enumeration consists in weighing out three milligrams (or 0.003 gramme) of flour, dividing this into five portions on as many microscope slides, wetting with water, covering with cover slips, and then counting under the microscope the numbers of hairs, epicarp and seed-coat particles in the five slides. The numbers given in the tables are those actually counted. To obtain the corresponding numbers in one gramme of the flour, these must be multiplied by 1000 and divided by 3, since 3 milligrams are taken.

TABLE I. *Numbers of microscopic offal particles in the different machine fractions or "stocks" from a milling of wheat contained in 3 milligrams of each stock.*

Machine	Hairs	Epicarp	Seed-coat	Total
A	Nil	Nil	Nil	Nil
B	"	"	"	"
C	"	"	"	"
D	5	"	"	5
E	7	"	"	7
F	13	5	"	18
G	18	26	"	44
H	25	26	"	51
Break (1, 2, 3)	28	24	3	55
Break (4)	38	30	13	81

The value of the method was next tested by applying it to the investigation of the "first" or "superfine" and the "second" or

¹ Bacteriologist to the County Council of Lanark.

"standard" quality of four well-known commercial brands of flour. The trade names of these are not given here, they are simply indicated by four numbers.

TABLE II. *Differences in first and second quality flours.*

	Flour	Hairs	Epicarp	Seed-coat	Total
No. 1	First	1	Nil	Nil	1
	Second	39	13	5	57
No. 2	First	Nil	Nil	Nil	Nil
	Second	75	13	1	89
No. 3	First	6	Nil	2	8
	Second	22	7	1	30
No. 4	First	Nil	Nil	Nil	Nil
	Second	23	10	7	40

This table shows clearly the difference between first- and second-quality flours. The information so obtained was turned to the investigation of reputed first-grade superfine or patent flours obtained from mills where no "tinting" is used, and from mills where bleaching was known to be carried out, or where the higher content in nitrites suggested that the flour had been "tinted" or artificially bleached.

TABLE III. *Unbleached first-grade flours.*

Flour	Hairs	Epicarp	Seed-coat	Total
No. 1	Nil	Nil	Nil	Nil
No. 2	1	"	"	1
No. 3	2	"	"	2

All three of these flours are obviously shown to be *true* first-grade by this test.

TABLE IV. *Bleached first-grade (?) flours.*

Flour	Hairs	Epicarp	Seed-coat	Total	Nitrites per million
No. 1	Nil	Nil	1	1	0.98
No. 2 <i>a</i>	23	18	13	54	3.4
No. 2 <i>b</i>	20	12	3	35	4.7
No. 2 <i>c</i>	10	12	3	25	3.8
No. 3	17	6	2	25	3.8
No. 4	10	Nil	3	13	2.8
No. 5	18	8	11	37	1.2
No. 6	10	12	10	32	3.7
No. 7	13	5	10	28	2.1

Out of these seven brands of flour all of which are well known commercially, and sold as *first-grade flours*, it is evident that the first one alone is really a high class flour. The others had evidently been prepared from a very "long divide" by including products of lower machines which would have been impossible before the introduction of bleaching.

Dr Patterson sums up the results of his examinations as follows:—"The lower grade qualities are both higher in yellow colour and contain hairs, epicarp and seed-coat particles. The bleaching removes the colour of the mass of lower grade material as a whole, but these particles are left unaltered and form a guide to the microscopist that the admixture has been disguised to the naked eye, and to a simple lens, by the bleach. It is not claimed that the offal particles are bleached, but that they form an invariable accompaniment of lower grade yellow coloured flour which must be bleached before it can be added to the rest of the flour. Hence either a high quality bleached or unbleached flour does not contain such particles, but a low quality flour bleached to represent a high quality flour does contain these particles, and my experience is that the majority of so-called high class bleached flours on the market are flours which are mixtures and would be low grade without bleaching."

If these differences revealed by the microscope really do exist between first and second qualities of bleached flour, and if examination of so-called "first" qualities of bleached flours now on the market reveals the fact that these are in reality mixtures of first and second qualities, the question arises, in the interests of the public, whether legislation ought not to be introduced compelling the open labelling and selling of such flours as "Bleached Flours."

The demand for white flours had its origin in the fact that originally, *before* bleaching was introduced, the white flour represented the "cream of the wheat," and that which possessed the better flavour and baking qualities, and this has recently been attested on oath by competent bakers.

There is also little question that the majority of bakers and other competent judges would say that bread baked from first quality or "patents" was better than that baked from an entirely "household" quality. This proves that in the unmixed condition there is really a superiority in the first quality and one resting on other factors than colour. This must also hold in comparative degree between varying mixtures of first and second qualities, that is to say the more first quality

and the less second quality there is in a given blended flour the more valuable that flour must be. Now, on the admission of the advertisement, the bleaching process allows more second quality to be mixed with first quality. Bakers and the public are entitled to know that this increased admixture of second quality is being made in a given case by an artifice.

The case cannot be met under the existing Food and Drugs Act, which only provides that the article supplied shall be of the nature, substance, and quality of the article demanded.

This legal point was pressed in the case tried at Hamilton, and witness after witness was asked, and pressed to answer, whether he regarded bleached flour as "genuine flour" and as to whether it was anything else but "flour."

The real point is, that there are various qualities of flour, and that one quality should not be treated artificially so as to simulate another, without being branded as having been so treated.

The question is a serious one, because bleaching is becoming so universal that the decreasing number of millers who attempt to supply unbleached flour only are placed at a disadvantage, and the public as a whole are unaware of the true state of affairs. A precedent for legislation outside the provisions of the principal Food and Drugs Act exists in the case of butter and margarine, where two Acts of Parliament have been passed, one controlling the sale of margarine, and the other the conditions of manufacture and importation of butter, margarine, and milk-blended butter and admixture of margarine and butter.

It is almost universally admitted that margarine is a good and wholesome food, perhaps as much so as butter, but it is a much *cheaper* food than butter, and ought not to be admixed with butter and sold as "butter." Yet this could be done, and actually was done on a large scale, until adequate legislation was introduced for the prevention of the practice. Margarine must now be sold with a label on it, and handed to the customer labelled as such. In addition, in order to preserve it absolutely distinctive as a cheaper food, and to enable the analyst to follow the matter up, it has been enacted that not more than ten per cent. of butter fats shall be added to margarine. In this way the public are protected both in the sale and manufacture of margarine.

The case of flour presents many analogies, the only difference being that both "superfine" or "patent" and "standard" or "household" flour are manufactured from wheat, while margarine is made from other fats than those of milk. But the difference is only a superficial one, for

chemically these other fats are the same, as to over 95 per cent. of their mass, as the fats of milk. They are also just as nutritious, and the main differences are colour and flavour, just as they are between the two qualities of flour, and the breads made from them. Curiously enough, the aesthetic tastes in colour are the opposite way round in the two cases, the yellow colour is desired in the butter, and is a disadvantage in the superfine flour, and to add to the absurdity it is the *same* colouring matter in the two cases, a substance called "carotene," soluble in oils.

Now, second-quality flour is a good and nourishing food just as is margarine, but that is scarcely a sufficient reason why it should be bleached, mixed with first-quality flour and the whole sold as first-quality flour.

This is precisely what was being done with margarine and butter previous to legislation, the only difference being that the same colouring matter was being put in instead of being bleached out.

The excuse moreover was the same, the satisfaction of a public aesthetic taste, and it may be added that it is less questionable to add an undoubtedly harmless vegetable colouring matter like carotene than to bleach that same harmless colouring matter out with a chemical agent which in fairly small doses possesses marked injurious effects. It is a disputed point in the matter whether the small amounts of nitrites developed in the flour in the bleaching process are, or are not, actually harmful, and it is practically impossible at present to settle the question one way or the other. But no one has ever claimed that they do any good, and in our present state of knowledge it would certainly be better to leave them out and allow the harmless and possibly beneficial carotene to remain in.

For example, recent advances in medical knowledge have taught us clearly that an important disease, called beri-beri, common in eastern countries amongst those sections of the population who subsist almost wholly on rice, is due to the removal of quite minute traces of substances in the outer layer of the rice grain which are a necessary constituent of the food. The individual who is better off and can afford to eat a varied diet can eat polished rice with impunity because he obtains this necessary constituent from other sources in his varied diet, but the poor coolie, if he substitutes an almost entire diet of polished rice for his less aesthetic "paddy," which contains portions of the outer layer attached to the endosperm, often suffers from beri-beri.

We do not as yet know whether carotene may not possess an important function in a very restricted diet. It is closely allied to, if

not identical with, a yellow pigment found in the blood serum and in nearly all the cells of the blood. It is found in the majority of the foods we eat on a mixed diet, and so the person who can afford a liberal and varied diet can afford to dispense with it in his bread. But in milk it is only present in the fats or cream globules, and when it is remembered that the children of the poor in our community are largely brought up on milk impoverished in fat, and upon bread, it is questionable whether we do wisely in allowing the carotene to be bleached out of the flour from which their bread is to be made.

Knowledge is imperfect in these matters, and, until it is more complete, artificial interference with a staple food supply and its treatment for commercial purposes with chemicals, should be scrupulously watched and guarded.

It is interesting to observe in regard to legislation what has been done in other countries, and we may note here the action taken by one of the greatest flour producing countries of the world, the United States of America.

The following is a statement of Food Inspection Decision¹ (No. 100) issued by the Board of Food and Drug Inspection of the United States Department of Agriculture, December 10th, 1908 :—

"Flour bleached with nitrogen peroxide² as affected by the Food and Drugs Act of June 30th, 1906, has been made the subject of a careful investigation extending over several months.

"A public hearing on this subject was held by the Secretary of Agriculture and the Board of Food and Drug Inspection, beginning Nov. 18th, 1908, and continuing five days. At this hearing those who favoured the bleaching process and those who opposed it were given equal opportunities to be heard.

"It is my opinion, based upon all the testimony given at the hearing, upon the reports of those who have investigated the subject, upon the literature, and upon the unanimous opinion of the Board of Food and Drug Inspection, that flour bleached by nitrogen peroxide is an adulterated produce under the Food and Drugs Act of June 30, 1906 ;

¹ This Decision seems still to be in force, but on account of the result of the legal proceedings in a case brought before the Courts in June, 1910, it is meantime practically in abeyance.

² It is now admitted, whether produced electrically or not, that the bleaching agent is nitrogen peroxide although, so late as Jan. 1912, the Flour Oxidising Co., Ltd. claimed in their advertisement that "The Corona produces electrified air of the finest quality." "The Corona is a natural process—only the elements (electricity and air) are used." *Milling*, Jan. 13, 1912.

that the character of the adulteration is such that no statement upon the label will bring bleached flour within the law; and that such flour cannot be legally made or sold in the District of Columbia, or in the Territories, or to be transported or sold in interstate commerce; or be transported or sold in foreign commerce except under that portion of Section 2 of the law which reads:

".....*Provided*, That no article shall be deemed misbranded or adulterated within the provisions of this Act when intended for export to any foreign country and prepared or packed according to the specifications or directions of the foreign purchaser, when no substance is used in the preparation or packing thereof in conflict with the laws of the foreign country to which said article is intended to be shipped.....

"In view of the extent of the bleaching process and of the immense quantity of bleached flour now in hand or in process of manufacture, no prosecutions will be recommended by this Department for manufacture and sale thereof in the District of Columbia or the territories or for transportation or sale in interstate or foreign commerce, for a period of six months from the date hereof."

JAMES WILSON,

Secretary of Agriculture.

WASHINGTON, D.C., *December 9, 1908.*

In other words, bleached flour is not to be supplied under any conditions to the American citizen, but can be sent abroad to those foreign countries whose laws allow it to be dumped upon them—of these, our own country is one. In contrast to this pronouncement is the judgment of Sheriff Shennan in the case heard at Hamilton, Lanarkshire, at the instance of the County Council of Lanark.

The decision given was in favour of the defendants, although Sheriff Shennan found that the flour in question had been bleached by nitrogen peroxide, and the conclusion of his summing up may be quoted:—"As I stated at the outset, this is not a matter of pure science. My natural sympathies are all with the prosecution. I do not see why these artificial changes should be produced in articles of food for aesthetic reasons. I appreciate the medical stand-point that where there is even the apprehension of danger because you are working with materials which under certain circumstances may produce noxious results, such processes should be prohibited. But such operations do not necessarily involve an offence against the Sale of Food and Drugs Act. My decision here is not based on the smallness of the amount used. As

was pointed out, an infinitesimal amount of formalin will produce deleterious chemical changes in milk. My judgment is rested in this, that adequate proof has not been tendered of deleterious results due to bleaching the sample of flour before me. I am afraid I must hold that the prosecution is based on apprehended evils rather than on proved deterioration. It will not do for the purposes of a prosecution to argue that the process should be prohibited, unless it can be proved that the effect is noxious. That would be an excellent argument to address to the legislature, but in these proceedings the Prosecutor must prove his case. I sympathise with the spirit which has induced the prosecution, and possibly its purpose may to some extent have been effected by the resulting publicity. But I come without any hesitation to the conclusion that the contravention alleged has not been proved."

The contravention alleged was that of the Section in the Act stating that "no person shall sell to the prejudice of the purchaser any article of food or any drug, which is not of the nature, substance, and quality of the article demanded by the purchaser." The failure of the Prosecution turned on not being able, with the quantities of nitrogen peroxide used commercially, to demonstrate unequivocally alteration in nature, substance or quality coming within the meaning of the Act.

The point of quality being inferior is discussed by the learned sheriff, who points out that there is no fixed standard for flour, and also that it had not been proved that the sample in question was of an inferior quality to that demanded. His Lordship was also of the opinion that other qualities of the different flours than colour appearing in the baking and use of the bread would in the general case prevent the substitution of lower for higher grades.

There is, however, here the direct evidence of Dr Patterson from the microscopic tests that in a large number of cases bleached flours sold as first-grade are in reality admixtures with second-grade. This, however, was held to be only general evidence, and it was not accepted as proven that the particular flour in court was of an inferior grade.

The question before us now in this paper is not that of a particular flour, but the question of whether in general the bleaching process allows lower priced grades to be mixed with higher, and on this point there exists not only the evidence of Dr Patterson, but the admission of the advertisers of the process themselves that bleaching gives the miller "a longer divide," which in plain English means enables him to admix more lower priced products into his superfine flour.

The present Food and Drugs Act does not touch this position, because this mixture is still "flour" and held to be "genuine flour," and as to its being "superfine flour" that again is a variable standard. The only way the public can be protected against this artificial interference with a natural standard is by new legislation, enacting two things. First, the proper labelling of bleached flour, and secondly, the regulation of the manufacture and bleaching of flour, in the same way as the manufacture of margarine is now regulated. If this were done, it would be quite certain that no bleached superfine flour would be manufactured in future, because with its label on it could not compete with the natural "cream of the wheat," and secondly, that portion of the British public which required whiteness in a lower class product would soon realize that it was obtained by artificial bleaching, and if it still used it from a desire to ape the superior article would do so knowingly, and not in ignorance as at present.

The contention that there are other differences between superior and inferior flour which appear in the baking and in the flavour and quality of the bread, and can act as a natural remedy against substitution of the inferior for the superior after bleaching, is not a valid contention against the introduction of protective legislation. Otherwise, if the superior article can stand on its own merits, there would be no need for legislation whatever, and all work against adulteration of food might forthwith be suspended.

Butter has superior qualities in the cooking, and flavour in eating, as against margarine, yet before the introduction of special legislation in this instance, margarine and butter were mixed and the product sold as butter, and the public were deceived in the matter and paid more than the proper value.

In an exactly similar manner, if our evidence given above is worthy of credit, inferior priced flour is mixed with more expensive flour, the product bleached before or after admixture, and the mixed product is sold as "superfine flour," "patents" or "firsts" and at the price of these dearer commodities.

In our opinion, therefore, and on a purely economic basis, there never was a clearer case proven for special legislation in the public interest.

Finally, it may be added that allowance here of artificial interference with this most important of our foodstuffs opens the door to other artificial chemical treatments of foods on the pretext of bettering them and catering for the public taste.

This is shown by the more recent introduction of chemical "improvers," which are now being added to flour in a wholesale fashion all over the country. This question is at present perturbing the whole milling and baking industries, although it has not yet come to wide public knowledge.

We have recently examined several samples of commercial flours which were found to contain as much as ten ounces of added "acid phosphate" to the sack.

The excuse is that a better "rising" flour is obtained in this way, so yielding from the same quantity of flour a larger loaf or scone. It is said by those who have "improvers" to sell, that addition of "acid phosphate" only gives in "weak" flours, that is those which rise poorly, the same good natural qualities that are found in good, naturally strong, flours. It is claimed that the substance added is one always present in wheat which happens to be deficient in the "weak" flours. This argument strongly recalls that of those who bleach flours, namely, that nature is only being imitated by an artificial ageing brought about by the bleaching, and that all that is being done is the hastening of a quite natural process.

Even if these statements be accepted—and they are highly questionable in both cases—does it follow that these artificial products should be sold as the natural articles? The commercial problem is here undoubtedly that of obtaining a higher price for a naturally lower-priced article by adding or taking away something which distinguishes the two. If this is to be allowed at all, the process ought to be publicly known and recognized, and the artificial product should be properly labelled and branded in a distinctive way. Also, if there be, as there undoubtedly is, danger of excess in the matter, the process of manufacture should be properly supervised and regulated by law, as is done in other similar cases.

Chemical Alterations in Flour caused by the Bleaching Agent.

Nitric oxide (NO) has no bleaching action upon flour, it only acts after it has taken up oxygen and formed nitrogen peroxide (NO_2). This gas in presence of water decomposes and forms nitrous and nitric acids in equi-molecular proportions. The bleaching probably arises from the action of the nitrous acid upon the colouring matter, carotene, of the flour. This substance is present only in very small quantity. It is present in less amount than is sufficient to react with the whole of

the nitrogen peroxide used commercially for bleaching, although that is also but a small quantity. As a result it follows that the excess of nitrogen peroxide, or the nitrous and nitric acids formed by the action of water in the flour upon it, must combine with the organic and inorganic constituents of the flour to form nitrites and nitrates.

In commercial bleaching an amount varying from one-third up to nearly all the colour is bleached out, according to the market for which the flour is intended. The recent work of Monier-Williams proves that with a strength of bleach removing about one-third of the colouring matter, not more than one-fourth of the nitrogen peroxide employed unites with the colouring matter, the remaining three-fourths must accordingly unite with other constituents of the flour.

This has been an important point to settle, as it now has been settled by these important experiments of Monier-Williams, for it was actually contended by experts examined in the trial at Hamilton, that the colouring matter alone was attacked by the nitrogen peroxide added, and that the other constituents were left entirely unaltered. This view was accepted by the court although it is contrary to all chemical theory.

When any active chemical substance is added to a mixture of several ingredients upon which it can act, it is a well-known and universal application of the fundamental chemical law of "Mass action," that the active agent will react, and distribute itself amongst these several ingredients in proportion to the product of its affinity for each and the amount of that constituent present, and it would be a breach of all chemical law that when present in insufficient quantity to satisfy all of them, it should unite exclusively with one and leave all the others wholly untouched. This however was argued in court and accepted by the court, and undoubtedly produced an effect in the finding arrived at.

It is necessary in order to study effectually the action of nitrogen peroxide upon the other constituents of flour, such as the proteins and fats, and to obtain definite workable alterations, to employ considerably larger quantities of nitrogen peroxide experimentally than are commonly used commercially.

This is a perfectly legitimate proceeding, quite valid in scientific work and one which has been used in other cases of the addition of noxious or poisonous substances to foodstuffs. For example, the deleterious action of formaldehyde upon foodstuffs can only be demonstrated with many times larger amounts than would be allowed in any article of food.

Yet this method was discredited as a "method of exaggeration" by the learned sheriff (quoting a witness for the defence) which could not legitimately be used as a process of proof.

The inference of the prosecution, on the other hand, was that the chemical methods of detection of change in these constituents (proteins and fats) were not sufficiently delicate to demonstrate them at the level of commercial use of nitrogen peroxide, but that they could be demonstrated clearly at higher amounts of nitrogen peroxide (although still small amounts), and therefore the logical inference was that they occurred in diminished degree with the smaller quantities. Probably few chemists would dispute this inference.

It was claimed, however, by the defence that a chemical substance at high dilutions often acts differently from its action at greater concentrations, and hence it might be, that at the very high dilution of chemical bleaching of flour the nitrogen peroxide acted only on the colouring matter, and left the other constituents quite untouched. In fact, figures provided by prosecution witnesses were extra-polated down to weaker concentrations, in an attempt to prove that this actually occurred.

The case selected by the defence to illustrate the fact that a substance may have different actions at different dilutions was a very curious one. It was claimed that the charring of sugar by strong sulphuric acid which does not occur with diluted acid was a parallel case, and this was apparently accepted by the court.

There is, however, nothing in common between the two types of reaction. The concentrated sulphuric acid acts when free from water as an energetic dehydrating agent, and when previously supplied copiously with water it obviously cannot have this action. Neither at the commercial bleaching strength, nor at that used in the experiments produced in proof by the prosecution, has nitrogen peroxide such an action, and in both concentrations nitrogen peroxide is being used as a highly diluted reagent. The true parallel would have been that of two different but high dilutions of sulphuric acid upon cane sugar, as compared with two different high dilutions of nitrogen peroxide upon flour. Diluted sulphuric acid has, as is well known, an effect upon cane sugar, in that it hydrolyses it into dextrose and laevulose, and no one has ever claimed that at a certain point, short of zero, this *entirely* ceases, which is the claim put forward in the case of nitrogen peroxide and flour.

In view of this point it is exceedingly interesting that the more

recent experiments of Monier-Williams have now clearly demonstrated that the greater part of the nitrogen peroxide is actually taken up by other constituents than the colouring matter. In fact, in order to reach the colouring matter at all, as was pointed out in court, the nitrogen peroxide must first be absorbed by the oil of the flour in which the colouring matter is dissolved. For this oil it possesses a high affinity and it is unthinkable that some of it should not combine with the oil.

The so-called "method of exaggeration" is accordingly a perfectly valid one to employ for studying the reaction of the flour constituents to the bleaching reagent, and one which is often used in other cases of similar study, and it may logically be assumed that these changes, although of course in lessened amount, occur to these constituents in the commercial process of bleaching.

By the use of these methods it is found that the chief constituents altered are the colouring matter, the fats, and the proteins, and these will now be separately considered.

Changes in the Colouring Matter.

The colouring matter of flour belongs to a class of yellow coloured pigments which are very closely related chemically and are found widely distributed in both the plant and animal kingdom. These yellow pigments are known as "luteins" or "lipochromes," and chemically they are highly unsaturated compounds, which form colourless addition products either with oxygen or nitrogen peroxide. The latter substance does not appear simply to oxidize them catalytically, but to form addition compounds with them. This is an important point since it shows that artificial bleaching and slow oxidation in the air are essentially different processes, as will be pointed out later.

The yellow pigments of this group are insoluble in water, but are soluble in oils, and in protein containing solutions such pigments are present in the animal kingdom in egg-yolk, blood serum, body fats and milk fats, and all oils of animal origin. In the vegetable kingdom they occur in all vegetable fats, flowers, seeds, fruits, and many other plant tissues.

One of the commonest "luteins" or "lipochromes" is the substance called "carotene," from having been isolated in pure condition from the carrot (*Daucus carota*); it occurs in many seeds, fruits and flowers. The amount of colouring matter in flour is excessively small, amounting according to Monier-Williams in unbleached flour to only two parts in

a million or less, and for this reason it is impossible to separate workable quantities from flour, but a close comparison of the two absorption spectra as carried out by Monier-Williams clearly shows its identity with carrotene, and spectroscopic and colorimetric comparisons with pure carrotene solutions enables its amount in flour before and after bleaching to be determined with accuracy.

By the use of these methods, Monier-Williams found that flour before bleaching contained 1.4 to 2 parts per million of carrotene and after the degree of bleaching used for the London markets contained 0.9 to 1.3 parts per million, so that roughly one-third of the carrotene was decolorized by the bleaching process. The increase per million in nitrites after bleaching amounted in these cases to about 1.2 parts per million, and as the quantity required for combination with the bleached carrotene only amounted to approximately 0.33 part per million it becomes obvious "that only a small proportion of the nitrogen peroxide used is concerned in the actual bleaching."

When flour is exposed to the air in thin layers, it takes up considerable amounts of nitrites and at the same time becomes whitened, though never so much so as when bleached with nitrogen peroxide, although the amount of nitrite acquired, especially in a vitiated atmosphere, may be considerably more than after moderate bleaching with peroxide. For example, experiments made in connection with the Hamilton case showed that thin layers exposed for some weeks acquired no less than 11 parts per million, calculated as sodium nitrite.

It is important to consider this air effect carefully, because of the argument that nitrogen peroxide bleaching is the same as natural air bleaching. The evidence may therefore be briefly summarized.

1. Flour can be whitened in pure air, without increase of nitrites, and Monier-Williams has shown that carrotene exposed to air bleaches with uptake of oxygen and without acquiring nitrite. Moreover, when carrotene is bleached with nitrogen peroxide, the colourless product produced is an addition compound with nitrogen peroxide and not one with oxygen, so differing essentially from the natural oxidation compound formed in air.

2. When flour is allowed to stand in air the bleaching effect is much smaller than corresponds to the nitrite uptake as shown by the experiments of Thomson made in the Hamilton case.

3. There is no evidence that free nitrogen peroxide exists in air. The rain water even of town districts is alkaline in reaction and there is always more than enough ammonia to neutralise any acids present,

so that there is no evidence that the nitrite taken up is taken as nitrogen peroxide, or produces any bleaching effect. All the tests employed and supposed to show nitrogen peroxide in air would be given equally by traces of combined nitrites, or by ozone. So that there is really no valid evidence of nitrogen peroxide bleaching by exposure to the atmosphere. On the contrary pure carotene exposed to the air in samples at London (and even at Widnes, Lanes.) by Monier-Williams showed not a trace of nitrite to the most delicate reagent (Griess-Ilosvay reagent) even after two months exposure, and did not contain nitrogen in any form.

Although this increase of nitrites on exposure to air has nothing in all probability to do with natural bleaching, it is important, in regard to judging from the nitrite content, whether the flour has been bleached in any given case. The amount taken up will be increased when the flour is exposed for sale in small packets but even then it is only a skin-layer which is affected, and it is probable that anything under commercial conditions lying above one part per million calculated as sodium nitrite indicates artificial bleaching. The figure is placed somewhat higher by Monier-Williams, namely at 1.5 to 2 parts per million, and from the large series of figures accumulated by Clark, and by Thomson, each independently checking the other, it may be said that this is really a maximum limit.

It scarcely needs pointing out, however, that the effective legal check would be a prohibition, or regulation, of bleaching at the mills, much more than relying on after-detection in purchased samples, where legal points can be raised over period and manner of storage, so that much vexatious litigation might be produced by using any arbitrary standard.

Moreover, as shown both by our own experiments and those of Monier-Williams, the nitrite content of highly bleached flours decreases, while that of unbleached flours increases, which again interferes with the setting of a standard maximum allowable content of nitrite.

It is the process of bleaching and all it involves which requires regulation, the amount of nitrite while an important matter in itself is secondary to this.

Effects of Nitrogen Peroxide on Oils or Fats.

Unsaturated fats and lecithins combine with nitrogen peroxide with great avidity to form addition compounds containing nitrogen. The gas is rapidly absorbed by the oils or fats in the cold, and a very large volume can be taken up. The properties of the fats and oils are

entirely changed in the process, as is shown by the following experiments:—

Experiment I. A quantity of olive oil, weighing 18.1 grammes, was introduced into a Winchester quart bottle, provided with a double bored rubber cork and exhausted of air. Nitric oxide gas (NO) and oxygen in the proper proportions to form nitrogen peroxide (NO_2) were then admitted, and the peroxide was found to be absorbed almost as soon as formed, until about 3870 c.c. of nitric oxide and 1850 c.c. of oxygen had been taken up. The oil acquired a green colour from dissolved nitrogen peroxide, in addition to that chemically combined, but on removing the altered oil, and heating the mixture, the dissolved peroxide was driven off leaving a pale yellow product. The physical properties were changed from those of olive oil to a thick viscid semi-solid mass. The weight had increased by approximately four grammes. The oil constants of this addition product were determined when the following changes were found:—saponification value = 325, that of the olive oil being 184; iodine value, less than zero, that of the olive oil being 87. This effect of an apparently negative iodine value arose from NO_2 set free having decomposed potassium iodide in the titration, for the control required 20.5 c.c. of thiosulphate solution, while the altered oil required 22.3 c.c.

It is thus obvious that the reagent used in flour bleaching is one which attacks and completely alters oils, and this to a small extent must occur in commercial bleaching, although it cannot be directly detected. The effects of continued uptake of minute quantities of organic nitro-bodies is unknown, and it would be difficult, or even impossible, to demonstrate such effects of continued use of small amounts, but it is known in pharmacology that organic nitro-bodies, such for example as amyl nitrite and nitro-glycerine, are most powerful agents.

Experiment II. A quantity of lecithin prepared by the usual methods from egg-yolk, and weighing 20.7 grammes, was similarly saturated with nitrogen peroxide, and took the gas up greedily. The weight increased from 20.7 to 23.6 grammes and the semi-fluid lecithin had changed to a solid substance. The original iodine value of the lecithin was 77.5, and this changed to 16.6, showing that nearly all unsaturated groups had become occupied by peroxide of nitrogen, and that the lecithin had become nitrated. The saponification value, which had been 276, changed to 341. Thus, again, the lecithin like the olive oil has been profoundly changed by the bleaching gas.

Experiment III. In this experiment a flour known to be unbleached was taken. This flour was guaranteed by the millers to be unbleached and this was also attested by its low content in soluble nitrites as shown by the Griess-Ilosvay test, namely 0.3 part, as sodium nitrite, per million. Bleaching operations were carried out on this flour by introducing known quantities into the Winchester quart as described above, and then bleaching with known volumes of nitrogen peroxide gas to the kilogram of flour. Bleaches were used of 20 c.c., 30 c.c., 100 c.c., and 4500 c.c. to the kilogram of flour, the latter high quantity being used to develop the full effect of nitrogen peroxide on the flour, and clearly demonstrate its nature.

The results shown below indicate that the smaller amounts only differ in degree of action from the larger quantities, and qualitatively act in the same fashion.

After bleaching the flour, the fats were extracted from weighed samples of each degree of bleaching, by thorough extraction with ether, and the constants determined in each case, as well as the percentage of nitrogen. The results are shown in the following table:—

Constants of flour-oils before and after bleaching.

	Bleach per kilogram	Nitrogen, percentage	Iodine value	Saponification value	Free fatty acid, percentage
1	Unbleached flour	0.68	111.4	200	10.8
2	Bleached with 20 c.c.	0.74	107.4	174	9.9
3	„ „ 30	0.82	96.5	155	8.5
4	„ „ 100	0.97	85.8	162*	7.6
5	„ „ 4500	1.26	82.5	245*	28.5

* These subsequent rises in saponification value with high degrees of bleach are due to nitrous acid in previous combinations with the fat being split off and neutralizing alkali.

Regarding colour of the extracted fat this is orange yellow in the extract from the unbleached flour, and the colour is diminished in the successive bleachings up to 100 c.c.; above this value the fat is browned by nitration products.

This experiment clearly shows that at these values of bleaching, the fats are altered, the degree of alteration increasing with the dosage. If it is to be claimed that the concentration of nitrogen peroxide used is higher than that employed in commercial bleaching, it may be answered that although lessened in quantity the results must qualitatively be the same at the lower level, although they pass into a region where they cannot be followed experimentally by methods of the delicacy lying

at our disposal. But there is no doubt on all chemical analogy that persons who consume bleached flour are daily using small amounts of nitrated fats, and the action of these, even in small quantities used over periods of years, is a new factor the value of which in metabolism is at present unknown to us, and it may be legitimately asked, for what reason are we compelled to submit to this unknown risk, as we all are being submitted to it without our knowledge and consent?

The nitration of the fats is shown in the table above most clearly by the two columns particularly dealing with percentage of nitrogen, where the increase shows nitration, nitrogen locked into the molecule of fat by the bleaching, and by the iodine value, where nitrogen peroxide entering and nitrating leaves less place for iodine to combine when the iodine value is determined.

Effects of Nitrogen Peroxide on the Proteins of Flour.

It is well known that both nitrous and nitric acids combine with proteins and alter them so that they become impossible as foodstuffs, and since nitrous and nitric acids are formed when nitrogen peroxide comes in contact with water, and there is water in flour, it would seem inevitable *prima facie* that the exposure of flour to nitrogen peroxide gas must alter the proteins of the flour. This is also shown quite clearly by the experiments recorded below upon unbleached flour as compared with the same flour after varying degrees of bleaching. As in the case of the oils described above, the degree of alteration is small at the commercial level, but even a small amount of nitration of the proteins is undesirable, and it may again be asked why it is carried out, as there can be no pretence that it is for the good of our health?

In order to determine the effects of bleaching with known amounts of nitrogen peroxide upon the proteins of flour, an unbleached flour was taken, and, as in the case of the experiments on the fats, this was treated with volumes of 20, 30, 100 and 4500 c.c. respectively.

Two main effects were discovered which are really closely inter-related. First, the degree of acidity of reaction to indicators such as phenol-phthalëin is increased, and secondly, the amount of the flour protein soluble in water is increased at the expense of the insoluble protein or gluten. This latter result does not really mean that the gluten is changed into soluble protein, in the lower bleaches at any rate, but gluten has its water solubility increased by the presence of acid, as shown by Hardy, up to a given point above which degree of acidity it

again decreases. The feeble bleaches alter the reaction in that direction which increases solubility. There is, however, along with this effect a change in the properties of the gluten, especially with higher quantities which show that it is attacked, and such changes undoubtedly occur in lessened degree at the lower bleaching strengths.

The acidity, mentioned above, is not due to free inorganic acids, but to acid salts, and salt-protein combinations, for which phenol-phthalëin is a most delicate indicator. Unbleached flour possesses, as shown by an aqueous extract, a reaction lying to the alkaline side of the neutral point, and the figures quoted as acidities only mean reactions to this particular indicator. The results show that bleaching with peroxide gives a disturbance towards the acid side.

The gluten may be separated from the starch and the soluble proteins by placing a weighed quantity of the flour on damped muslin, kneading into a dough with a small quantity of water, and then washing the excess of starch away through the muslin. The amount of insoluble protein or gluten may then be determined by estimating the amount of nitrogen by Kjeldahl's method and multiplying the nitrogen figure by a factor (usually taken at 5.8) which expresses the ratio of gluten weight to the nitrogen contained in dried gluten. The soluble protein may be determined in the aqueous extract by a similar method. It was by such methods that the figures given in the table below were obtained.

When 30 c.c. or over of nitrogen peroxide per kilogram is used as a bleaching agent upon flour, there arise changes in the gluten obtained by the method given above which are quite obvious to sight and touch, and although these are not visible when 5 c.c. or 10 c.c. are used, it is only because of diminished degree of action, for the total amount of nitrogen as nitrite and in combination with the colouring matter and fats is much below that known to be added in the bleaching process, so it is obvious that some must be in union with the protein. The affinity of protein for the reagent is well known. When less than 30 c.c. of peroxide is used, the amount of unchanged gluten disguises and conceals the smaller altered amount, but all chemical theory indicates that some portion must be altered at the lower bleaches similarly to what becomes manifest in the mass, when a greater quantity is changed or affected by the acids formed by the action of the peroxide.

Gluten as obtained from unbleached flour is a tenacious, stringy substance of a pale yellowish colour, since it contains enmeshed in it

the oil and colouring matter. It resembles in its physical properties the fibrin of blood, and contracts in an elastic way like fibrin. It is this body which is so valuable in the baking and raising properties of flour in bread making, and distinguishes wheat flour as superior to all other ground-up cereals. It is a kind of natural rubber which coats the bubbles formed in the bread with a fine pellicle, holds the bread together and gives the fine skin of the bread.

Now nitrogen peroxide in sufficient quantity completely destroys these springy and elastic qualities, and gives an altered gluten which has no strength, does not stretch but breaks easily across, and in part dissolves and passes through the muslin.

Of course, in commercial bleaching nothing approaching this degree of alteration is produced, or the process of bleaching would have been killed in its infancy, for bread could not be baked with such a product. But an incipient degree of such a change must occur even with small quantities, and evidence was given at the Hamilton trial showing that the tenacity of bread made from bleached flour was not so great as that from unbleached and that the skin was not so excellent.

Additional evidence, if such were required, that change in lessened degree occurs with the smaller quantities of peroxide is given by the figures for increased soluble protein and diminished gluten in the experiments now to be quoted.

Experiment I.

Sample		Acidity to phenolphthalein in e.c. of $\frac{N}{100}$ alkali per 100 grammes of flour	Gluten percentage	Soluble protein percentage
1	Unbleached flour	64	1.40	4.67
2	Bleached flour 100 c.c.	84	0.49	5.05
3	„ „ 4500	1000	0.34	5.24

In addition to the gluten and soluble protein there is of course a certain amount of insoluble protein mixed with the starch which passes through the muslin.

Experiment II. In this experiment the gluten was not separated off. The sample of flour was simply thoroughly mixed up with a measured volume of water, viz. one of flour to ten of water, and allowed to stand with occasional agitation for 12 hours. The samples tested and results were as follows:

Sample			Acidity to $\frac{N}{100}$ alkali with	Soluble protein percentage
			phenol-phthalein as indicator	
1	Unbleached flour		108	1.20
2	Bleached flour	20 c.c.	124	1.25
3	" "	30	126	1.27
4	" "	100	136	1.48
5	" "	4500	988	2.40

CONCLUSIONS.

1. Bleached flour is not known to be bleached by the great majority of those who consume it.

2. There does exist a demand for whiteness in flour, and previously to the advent of bleaching this was based on a real difference between white superfine flour and the cheaper yellower flour called "household" or "bakers" flour.

3. The difference consists in this, that the superfine contains the ripest and best part of the flour or "cream of the wheat," while the lower grade consists of less ripe or less developed endosperm and is richer in oil which contains the colouring matter carotene, and so is yellow in colour.

4. Bleaching by decolorizing the carotene removes a criterion of quality between the two grades of flour and allows the cheaper quality to be admixed with the dearer, and the whole to be sold as first quality.

5. That this admixture is made possible is shown in two ways: first, the sellers of the bleaching apparatus advertise in milling journals that the process enables the miller to increase his "divide," and secondly, there are minute microscopic particles of offal in the products of the lower machines which are not bleached or altered in the process, and which serve the microscopist as a guide to how the flour has been blended. Examination of commercial flours shows clearly that a large number of high-priced flours are such mixtures and could not be sold as such unless previously bleached.

6. Bleaching confers no advantage in nutritive properties or flavour upon the flour, and the large sum spent upon bleaching flour is really a national waste.

7. Bleaching flour with considerable amounts of nitrogen peroxide alters both fats and proteins by nitrating them. Although the changes at the level of commercial bleaching are small, there is no knowledge as to how the small amounts of organic nitro-bodies formed may affect the

human body in prolonged use for years, and as there is no counterbalancing advantage, and an addition also to the price obtained by simulating a superior article, it is suggested either that bleaching should be prohibited, or regulated and notified clearly by label to the purchaser.

8. Bleaching by nitrogen peroxide is not a more rapid achievement of a slowly occurring natural process, but is essentially distinct. For while natural whitening in pure air consists in an oxidation of the colouring matter, bleaching consists in the formation of addition compounds between nitrogen peroxide and the colouring matter.

ON THE AMERICAN METHOD OF STANDARDIZING TETANUS ANTITOXIN.

By ALFRED MacCONKEY.

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IN October 1907, the United States Government issued a circular defining the unit which should be used for measuring the strength of tetanus antitoxin.

Details of the method of standardization in which this unit is used were published by Rosenau and Anderson in Bulletin No. 43 of the Hygienic Laboratory of the Public Health and Marine Hospital Service.

It is claimed for this method that it is *simple, direct, and accurate*—attributes, which, if proved true, would compel its adoption universally. It seemed advisable therefore to study the method somewhat carefully and ascertain whether the claims made for it were well grounded.

The UNIT is defined as “ten times the least quantity of antitetanic serum necessary to save the life of a 350 gramme guinea-pig for 96 hours against the official test dose of a standard toxin furnished by the Hygienic Laboratory of the Public Health and Marine Hospital Service” and it is the *toxin*, in the form of a dry powder, and *not*, as in the case of the diphtheria unit, the antitoxin which is given out to manufacturers for the purpose of standardizing serum.

The L + or official test dose of the toxin at present issued contains just 100 M.L.D.'s for a 350 gramme guinea-pig.

The Bulletin carefully draws attention to certain points in technique which are considered of great importance for the success of the method. They are :—

- (1) *Diluting fluid for both toxin and antitoxin.*

A 0·85% solution of chemically pure sodium chloride sterilized by boiling.

- (2) *Time and temperature.*

The mixtures of toxin and antitoxin are kept 1 hour at room temperature in diffused light before injection.

- (3) *Amount injected.*

A total amount of 4 c.cm. of the toxin-antitoxin mixture is always injected into the guinea-pig.

- (4) *Concentration of the toxin.*
1 c.cm. of the toxin dilution = the test (or L+) dose.
- (5) *Concentration of the antitoxin.*
Dilution tables are given on p. 50 of the Bulletin.
- (6) *Weight of guinea-pig.*
The test animals should weigh 340-370 grammes.
- (7) *Site of injection.*
The injection is always given subcutaneously into the tissues of the abdomen about the level of the umbilicus.
- (8) *Time of death.*
The number of immunity units contained in the serum is determined from the amount given the animals that are *living* 96 hours after the inoculation of the mixtures.

We must now proceed to analyse some of the results given in the Bulletin.

On p. 7, it is stated that "the L+ dose is the smallest quantity of tetanus toxin that will neutralize 1/10th of an immunity unit plus a quantity of toxin sufficient to kill the animal in just 4 days" and in Table II, pp. 9 and 10, are given the results of injecting the L+ dose of toxin + 1/10 unit of antitoxin.

Tests were made on 19 occasions, on all but 2 of which two or more animals were inoculated on the same day. On only one occasion did the two animals die at the same time. The intervals between the times of death of guinea-pigs inoculated with the same volume of the same mixture on the same day varied between 0 and 74 hours. There was a difference of four days between the earliest and the latest death. Of the 42 animals used only three died in just four days, *i.e.* only some 7-8% died at the time specified in the definition of the L+ dose. Twenty-six died within four days (two under three days) and thirteen in over four days (one in over five, and one in over six days), *i.e.* about 57% died during the fourth day, and about 25% during the fifth day, or taking them together 83% died during the fourth and fifth days.

Again in Table IV, p. 16, are given the results of inoculating the M.L.D. of the standard test toxin into 58 guinea-pigs. Of these animals

	4 died during the 3rd day
21	" " " 4th "
6	" in 96 hours exactly
17	" during the 5th day
7	" " " 6th "
2	" " " 7th "
1	" " " 8th "

Thus only about 10% of the animals died at the time specified in the definition of the L+ dose; while 75% of them died during the fourth or fifth day. The longest interval between the death-times of animals inoculated on the same day was 3 days 23 hours.

We find then that 83% of the deaths in Table II and 75% of those in Table IV occurred during the fourth and fifth days and consequently it would seem that for practical purposes the death-time-limit may be taken as the fourth or fifth day.

It is necessary here to consider the meaning of the word "day" in this paper. From the American Bulletin one gathers that each period of 24 hours counting from the hour of inoculation is held to constitute a "day" and this definition is adhered to in all experiments detailed below, which refer to the *minimal lethal dose only*. When however it is a question of testing a serum, then the remaining hours of the day of inoculation are neglected and the first day commences on the morning after inoculation. This method of calculation is found to be most convenient from the point of view of routine work and does not lead to a serum being overestimated.

Now it is claimed (as stated above) for this method of standardizing tetanus antitoxin that it is simple, direct and accurate. As it is based on and is practically identical with Ehrlich's method of evaluating diphtheria antitoxin we may allow the first two claims and confine our attention to ascertaining whether the method is accurate.

The first point to determine is whether the test toxin is stable.

In the following Table I are given the results of the tests made to ascertain the minimal lethal dose of the standard dry powdered toxin.

This toxin has been sent on several occasions by Dr J. F. Anderson (I am pleased to have this opportunity of thanking him most sincerely for his kindness) and has been preserved in a vacuum desiccator in the ice chest. The powder was dissolved in 0.85% salt solution so that 1 c.cm. of the solution contained 0.000006 gm. of toxin. Each dose was made up to about 4 c.cm. with the same saline solution and was injected beneath the skin of the abdomen between the ensiform cartilage and the umbilicus.

A consideration of this table shows that this standard dry test toxin has been tested on 17 occasions during a period of 23 months and that 0.000006 gm.—the dose stated on the label to be the M.L.D.—of this toxin has killed 14 out of 17 guinea-pigs weighing 340–380 grms. on the fourth or fifth day. In other words 82% of the animals died within the specified time limits. This is quite in accordance with the results

TABLE I.

Examination of Sample No. 3 of the United States standard dry powdered tetanus toxin received March, 1911. The M.L.D. was stated to be ≈ 0.000006 gm. One guinea-pig was used for each dose. Weight of guinea-pigs, 340-380 grms. unless otherwise stated. The figures in the columns beneath the doses give the number of the 24 hour period, counting from the time of inoculation, during which the animal died or, where noted, first showed signs of tetanus.

Toxin 3.

Date of test	Dose								Remarks
	0.00001	0.000009	0.000008	0.000007	0.000006	0.000005	0.000004	0.000003	
6. 4. 11	4	3	did not kill	...	
"	5	5	7	6	8	...	Guinea-pigs, 445-470 grms.
8. 8. 11	4	4	4	5	
9. 10. 11	4	4	5	6	6	...	
2. 11. 11	4	4	4	6	6	...	
							slight tetanus		
29. 2. 12	6	4	4	3	4	...	Inoculated in back
"	3	4	4	5	6	...	" thigh
"	3	3	4	6	6	...	" abdomen
25. 6. 12	4	4	5	5	
24. 9. 12	4	5	5	6	7	...	
							marked tetanus		
21. 10. 12	4	4	7	5	no symptoms	...	
					distinct tetanus				
29. 10. 12	4	5	5	
8. 11. 12	4	4	4	5	6	...	Dilutions made with NaCl
"	4	4	4	5	6	...	Dilutions made with dist. water
"	4	4	5	5	5	...	Dilutions made with tap-water
							slight tetanus		
28. 1. 13	4	5	5	
							slight tetanus		
28. 2. 13	6	7	6	7	7	...	
				tetanus		tetanus	tetanus		
17. 3. 13	9	5	5	7	
	tetanus								
18. 4. 13	5	4	5	6	5	
							slight tetanus		

Toxin 4. Received July, 1912.

28. 2. 13	1	4	4	4
5. 5. 13	3	3	4	4	5

TABLE II.

The dry powdered toxin was dissolved in 0.85 % NaCl solution in such a proportion that 1 c.cm. contained 0.0006 grm. of toxin. Then 1 c.cm. of this dilution was added to 99 c.cm. of saline, distilled water or tap-water respectively, thus giving a solution of which 1 c.cm.=0.000006 grm. of toxin or 1 M.L.D. The doses were measured out into test-glasses and made up to about 4 c.cm. with saline, distilled water or tap-water as the case might be.

Test of Toxin No. 3.

Diluting fluid	Guinea-pig Weight	Grm. of toxin :				
		0.000008	0.000007	0.000006	0.000005	0.000004
0.85 % saline	365	4
	350	...	4
	350	4
	345	5	...
	345	6
Distilled water	340	4
	370	...	4
	365	4
	340	5	...
	340	6
Tap-water	340	4
	350	...	4
	350	5
	350	5	...
	340	symptoms of tetanus on 5th day

Test of Toxin No. 4. Received July, 1912. Tested February, 1913.

0.85 % saline	365	3
	350	...	3
	370	3
	380	3	...
	345	4
Distilled water	350	3
	345	...	4
	370	4
	365	5	...
	345	5
Tap-water	380	3
	350	...	3
	340	3
	360	4	...
	340	5

The numbers in the columns beneath the doses denote the day on which the animal which received that dose died.

given by Rosenau and Anderson and so we may accept this dry powdered toxin as a product which remains stable for 23 months when kept under proper conditions.

It is necessary to mention that the saline solution used in these experiments was made up with an ordinary "table salt" and not with chemically pure sodium chloride such as is recommended by the authors of the Bulletin.

This raises the question as to whether one might use distilled water or tap-water as the diluting fluid should it by chance happen that on a very urgent occasion the amount of saline available was not sufficient for all purposes.

With the object of furnishing an answer to this question two experiments were made, one with Toxin No. 3 and one with Toxin No. 4. The results are given in Table II.

From these results we conclude that once the dry powdered toxin has been dissolved in 0.85% NaCl solution we may use either distilled water or tap-water for the making of any further dilutions which may be necessary.

The next point to determine is whether this standard test toxin gives consistent values when used on several occasions to evaluate the same serum.

Now Rosenau and Anderson consider it most important that the total quantity injected each time should be exactly 4 c.cm. so that the pressure effects may be always the same. In Ehrlich's method of testing diphtheria antitoxin such accuracy is not considered to be absolutely necessary and the quantities to be inoculated are made up to *about* 4 c.cm. Experiments were therefore made to ascertain at the same time (1) whether the slight latitude allowable in testing diphtheria antitoxin would render the method valueless for the standardization of tetanus antitoxin, and (2) whether the standard dry powdered toxin gave consistent results with the same serum.

A serum, T. 26, was taken and a rough test made, using total quantities of *about* 4 c.cm.

The details of the test are given below.

¹ This analysis of the salt was kindly supplied by the manufacturers, Messrs D. Bumsted and Co., and was stated to be an average analysis:

Sodium chloride	99.28 %
Insoluble	0.02
Calcium sulphate	0.33
Magnesium chloride	0.05
Sodium sulphate	0.32

TABLE III. *Testing serum T. 26.*

Weight of guinea-pigs	Amount of toxin	Amount of serum (c.cm.)	Day : 1	2	3	4	5	6
370	0.0006 grm. U.S.A. 3	1/1500	—	S				
340	"	1/1600	—	..	—	—	—	—
340	"	1/1700	—	..	—	t	ttt	ttt
340	"	1/1800	—	..	?	ttt	†	
345	"	1/1900	—	..	ttt	†		
360	"	1/2000	—	..	ttt	†		

S=Sunday, no observations made. t=tetanus, the number of letters showing the intensity of the symptoms. †=death, the position of the cross indicates approximately the time of death. —=no symptoms.

Note. The animal's day begins at 6.30 a.m. and ends at 5.30 p.m. when the houses are closed for the night. Notes of the condition of the animals are made each morning between 9 a.m. and 10 a.m. A death taking place between 5.30 p.m. and 6.30 a.m. is indicated by a † between two days. Should death occur between 6.30 a.m. and 9.30 a.m. then a † is placed on the left-hand side of the space for the day. A note of the animal's condition shows that it was alive at say 9.30 a.m. After that the † is placed to indicate approximately the time of death up to 5.30 p.m. Full details of the experiments are given so that the reader may form his own opinion as to the value of the method.

These results give a value of between 180–190 units per c.cm.

The test was then repeated—using two series of animals. In the case of one series the dilutions were made as in the last test but in the case of series 2 according to the dilution tables given in the Bulletin.

In both series the total volume injected was not measured accurately but was *about* 4 c.cm.

TABLE IV. *Second test of T. 26.*

Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day : 1	2	3	4	5	6	Remarks
345	0.0006 U.S.A. 3	1/1650	—	—	—	t	S	ttt	
350	"	1/1700	—	—	—	tt	..	tt	Dilutions made
360	"	1/1750	—	—	t	tt	†		in way usual
360	"	1/1800	—	—	—	ttt	†		in testing diph-
380	"	1/1850	tt	†	accident during inoculation				theria antitoxin
380	"	1/1900	—	—	tt	ttt†	S		
365	"	1/1666	—	—	—	tt	..	tttt	
355	"	1/1739	—	—	t	tt	..	tttt	Dilutions made
350	"	1/1786	—	—	t	tt	..	tttt	according to the
365	"	1/1818	—	—	t	t	..	†	American tables
360	"	1/1850	—	—	tt	ttt	..	†	
360	"	1/1905	—	—	tt	†			

S=Sunday, no observations. —=no symptoms. †=death, the position of the cross indicating approximately the time of death. t=tetanus, the number of letters showing the intensity of the symptoms.

These results agree with those of the previous test, but to make quite certain the test was repeated. On this occasion the total quantity injected was made to vary considerably.

TABLE V. *Third test of T. 26.*

Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm)	Day :	1	2	3	4	5	Remarks	
365	0.0006	1/1650	—	S	—	—	—	tt	ttt	Doses made up to <i>about</i> 4 c.cm.
	U.S.A. 3									
355	"	1/1700	—	"	—	—	—	tt	tttt	
350	"	1/1750	—	"	—	—	tt	†		
345	"	1/1800	—	"	—	—	—	ttt	†	
345	"	1/1850	—	"	—	—	—	ttt	†	
375	"	1/1650	—	"	—	—	—	t	tt	Doses made up to <i>exact-</i> <i>ly</i> 4 c.cm.
370	"	1/1700	—	"	t	t	—	ttt	tttt	
360	"	1/1750	—	"	—	—	t	ttt	†	
370	"	1/1800	—	"	t	tt	—	tttt	†	
365	"	1/1850	—	"	t	tt	—	tttt	†	
375	"	1/1650	—	"	—	—	tt	tt	tttt	Doses made up to <i>exact-</i> <i>ly</i> 3 c.cm.
370	"	1/1700	—	"	?	—	tt	tt	tttt	
380	"	1/1750	—	"	—	—	ttt	tttt†		
370	"	1/1800	—	"	—	—	ttt	tttt†		
365	"	1/1850	—	"	—	—	ttt	tttt†		
<i>Doses</i>										
350	"	1/1650	—	"	—	t	—	tt	ttt	2.21 c.cm.
375	"	1/1700	—	"	?	—	tt	tt	tttt	2.18 "
345	"	1/1750	—	"	—	—	tt	tt	tttt	2.14 "
360	"	1/1800	—	"	—	—	tt	†		2.11 "
365	"	1/1850	—	"	—	—	ttt	tttt†		2.08 "
355	"	1/1666	—	"	—	—	ttt	†		Doses made up to exactly 4 c.cm.
370	"	1/1739	—	"	—	tt	†			
350	"	1/1786	—	"	—	—	tttt†			American tables of dilution used
350	"	1/1818	—	"	—	—	ttt	†		
375	"	1/1850	—	"	—	—	—	tttt†		

S=Sunday, no observations made. †=death. —=no symptoms. t=tetanus.

We see that the results of the first four series agree amongst themselves and confirm the results of the two previous tests. It is only the last series which is not concordant. It was therefore thought advisable to make a fourth test and to use total quantities of exactly 4 c.cm. in each case. (Table VI.)

From the result of this last experiment we must conclude that the discordant result which occurred in the third test of T. 26 should be

TABLE VI. *Fourth test of T. 26.*

Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day:						Remarks
			1	2	3	4	5	6	
340	0.0006 U.S.A. 3	1/1666	—	—	—	—	—	S tt	Dilutions ac- cording to the American ta- bles.
340	"	1/1739	—	—	—	t	t	" ttt	
365	"	1/1786	—	—	—	tt	tt	" ttt	
340	"	1/1818	—	—	—	—	t	" ttt	
340	"	1/1850	—	—	—	tt	tt †		
340	"	1/1650	—	—	—	—	—	S t	
360	"	1/1700	—	—	—	—	—	" t	
375	"	1/1750	—	—	—	—	—	" ttt	
365	"	1/1800	—	—	—	tt	ttt	" †	
340	"	1/1850	—	—	—	t	tt †		

neglected. All the others agree in measuring the antitoxin content of serum T. 26 at 185 units per cubic centimetre.

We have then found that the standard dry powdered test toxin, Sample No. 3, has given consistent results with serum T. 26 and that some latitude in the amount of the total quantity injected is permissible.

The question then arises whether another batch of toxin would give equally good results. A first answer to this question is given by the tests detailed in Table VII.

TABLE VII. *Evaluation of tetanus antitoxic serum T. 8.*

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day:						Remarks
				1	2	3	4	5	6	
28. 2. 13	350	0.0006 U.S.A. 3	1/1300	—	—	—	—	—	—	This test made with tetanus tox- in U.S.A. 3 gave a titre of 150 units per c.cm.
	340	"	1/1400	—	—	—	—	—	—	
	345	"	1/1500	—	—	—	—	—	—	
25. 2. 13	350	"	1/1500	—	—	—	t	S	ttt	
	340	"	1/1750	—	t	ttt †				
	340	"	1/2000	—	ttt †					
	340	"	1/2225	—	†					
	370	"	1/2500	—	†					
28. 2. 13	365	0.0006 U.S.A. 4	1/1100	—	—	—	—	—	tt	When tested a- gainst tetanus toxin U.S.A. 4 the titre was found to be 140 units per c.cm.
	355	"	1/1200	—	—	—	—	t	ttt	
	345	"	1/1300	—	—	—	tt	ttt	tttt	
	340	"	1/1400	—	—	t	tttt †			
25. 2. 13	340	"	1/1500	—	ttt †					
	340	"	1/1750	—	ttt†					
	340	"	1/2000	—	†					
	350	"	1/2250	—	†					
	370	"	1/2500	t	†					

From these results we may conclude that (1) Toxin 3 has deteriorated somewhat, or (2) Toxin 4 is a little stronger than it was supposed to be, or (3) 10 units is too small a difference to make between the doses of serum.

A consideration of Tables I and II suggests that, at the time the experiments given in Table VII were made, Toxin 3 had begun to go off and that the L+ dose of Toxin 4, supposed to contain 100 M.L.D., might contain more.

To obtain further information upon these points these toxins were tested against a liquid standard tetanus antitoxin kindly supplied by Messrs Meister, Lucius and Brünig. (Table VIII.)

We see that the results given by Toxin 3 are not so uniform as those given by Toxin 4 and they value the serum higher. Thus, according to Toxin 3 the serum contains from 170–242 units per c.cm. and according to Toxin 4 from 170–187 units per c.cm. (one cannot lay much stress on the test of 24. 4. 13 as the bottle of serum by this time was almost empty (it contained 1.5 c.cm.) and the serum was six weeks older), a difference in the one case of 70 units and in the other of 17. One is inclined to ascribe this difference to deterioration in Toxin 3, but one cannot be certain.

Experiments were then made with a dry standard tetanus antitoxin for samples of which I am much indebted to the great kindness of Prof. Ehrlich. (Table IX.)

From the tests of 1. 5. 13 and of 3. 5. 13, 0.0006 gm., which was stated to be the L+ dose of Toxin 4, is neutralized to the requisite extent by 0.25 c.cm. of the serum dilution used. This amount of serum was therefore taken as the test dose for varying quantities both of Toxin 3 and of Toxin 4. The results are given in Table X.

We find that 0.0006 gm. of Toxin 4 gives the same result as before and is equal to 0.0008 gm. of Toxin 3. It is probable therefore that the want of agreement in the results of tests with the two toxins is due to deterioration of Toxin 3 but whether entirely so is impossible to tell from these experiments. A little light may however be thrown upon this point if we analyse the tests made to ascertain the keeping qualities of our serum given in Table XI.

We see that some of these sera were tested against Toxin 3 alone at a time when there was no doubt that deterioration had not begun. Seven sera thus tested give an average loss in unitage of some 18% in 12 months whereas nine sera tested first against Toxin 3 (when at proper strength) and then against Toxin 4 show a loss of about 34% in

TABLE VIII. *Evolution of liquid standard tetanus antitoxin (Hoechst).*

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.c. of 1 in 425 dilution)	Day: 1	2	3	4	5	6	Origin and number of serum used. Remarks
12. 2. 13	340	0.0006	1	—	—	—	S	—	—	
		U.S.A. 3								
	355	„	1/3	—	—		..	—	—	
	340	„	1/10	t	†					
	360	„	1/50	tt	†					
19. 2. 13	340	„	0.33	—	—	S	—	—	—	
	340	„	0.3	—	—	..	—	—	—	
	340	„	0.25	—	—	„	—	—	—	
	340	„	0.2	—	—	„	t	t	ttt	
	355	„	0.15	—	tt	†				
28. 2. 13	340	„	0.2	—	S	—	t	tt	ttt	
	355	„	0.175	—	..	—	tt	†		
	355	„	0.15	—	..	ttt	†			
	340	0.0006	0.3	—	„		tt	tttt	†	
		U.S.A. 4								
	355	„	0.25	—	..		tt	†		
	350	„	0.2	—	..	†				
	355	„	0.175	—	..	†				
	340	„	0.15	—	..	†				
6. 3. 13	340	0.001	0.25	—	tt†					
		U.S.A. 3								
	355	„	0.2	—	†					
	350	„	0.175	—	†					
	340	0.0008	0.25	—	ttt	†				
		U.S.A. 3								
	345	„	0.2	—	ttt	†				
	365	„	0.175	—	tttt	†				
	340	0.0006	0.25	—	—	S	t	tt	tttt	
		U.S.A. 3								
	340	„	0.2	—	—	..†				
	340	„	0.175	—	tt	†				
	345	0.0006	0.25	—	—	S	ttt	†		
		U.S.A. 4								
	340	„	0.2	—	ttt†					
	340	„	0.175	—	ttt	†				
	350	0.0004	0.25	—	—	S	—	tt	tttt	
		U.S.A. 4								
	365	„	0.2	—	—	..	t	tttt	†	
	340	„	0.175	—	—	..†				
	340	0.0002	0.25	—	—	S	—	—	—	
		U.S.A. 4								
	340	„	0.2	—	—	..	—	—	—	
	340	„	0.175	—	—	..	—	—	—	

Hoechst standard serum 1:25 fact.
Serum diluted 1 in 425.

TABLE VIII (continued).

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.c. of 1 in 425 dilution)	Day: 1	2	3	4	5	6	Origin and number of serum used. Remarks
11. 3. 13	355	0.0007 U.S.A. 3	0.275	—	—	—	ttt	S	†	Hoechst standard serum 4.25 fach. Serum diluted 1 in 425.
	340	..	0.25	—	—	tt	†			
	340	..	0.225	—	t	†				
	380	..	0.2	—	ttt†					
	365	..	0.175	—	ttt†					
	345	0.0006 U.S.A. 4	0.275	—	—	—	—	S	—	
	340	..	0.25	—	—	—	t	..	†	
	340	..	0.225	—	—	t	ttt†			
	340	..	0.2	—	tt	ttt†				
	345	..	0.175	—	tt	ttt†				
24. 4. 13	340	0.0006 U.S.A. 1	1/1500	—	t	S	ttt	ttt†		Same serum. The aunts given in 3rd column represent the quantity of undiluted serum present in the mixture.
	340	..	1/1750	—	tt	†				
	340	..	1/2000	—	ttt†					
	340	..	1/2250	—	ttt†					
	340	..	1/2500	—	†					

TABLE IX. Evaluation of Frankfurt standard dry tetanus antitoxin.

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of solution of serum (c.c.)	Day: 1	2	3	4	5	6	Remarks
1. 5. 13	350	0.0006 U.S.A. 4	0.4	—	—	—	—	—	—	The contents of one tube of dry antitoxin were dissolved in exactly 26 c.cm. of 0.85 % NaCl solution.
	340	..	0.4	—	—	—	—	—	—	
	340	..	0.2	—	ttt†					
	340	..	0.2	—	ttt	†				
	340	..	0.133	—	†					
	340	..	0.133	—	†					
	350	..	0.1	—	†					
	345	..	0.1	—	†					
	365	..	0.088	t	†					
	350	..	0.088	—	†					
	340	..	0.066	tt	†					
	350	..	0.066	tt	†					
3. 5. 13	340	..	0.4	—	—	—	—	—	—	The contents of one tube were dissolved as before in exactly 26 c.cm. of 0.85 % NaCl solution.
	345	..	0.4	—	—	—	—	—	—	
	340	..	0.35	—	—	—	—	—	—	
	360	..	0.35	—	—	—	—	—	—	
	370	..	0.3	—	—	—	—	—	—	
	370	..	0.3	—	—	—	—	—	—	
	340	..	0.25	—	—	tt	ttt	ttt†		
	350	..	0.25	—	—	tt	tt	ttt		
	355	..	0.2	—	tt	ttt†				
	350	..	0.2	—	ttt	ttt†				
	370	..	0.15	tt	†					
	340	..	0.15	—	ttt†					
	340	..	0.1	ttt	†					
	340	..	0.1	—	†					

TABLE X. *Testing toxins 3 and 4 against the Frankfurt standard serum.*

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum dilution (c.c.)	Day:	1	2	3	4	5	6	Remarks
20. 5. 13	340	0.0008	0.25	—	—	tttt	†				
		U.S.A. 4									
	360	"	"	—	—	tt	ttt	tttt	†		
	340	0.0007	"	—	—	tt	tt	tttt	†		
	355	"	"	—	—	tt	ttt	†			
	350	0.0006	"	—	—	—	t	tt	S	ttt	
	350	"	"	—	—	—	t	ttt	"	ttt	
	360	0.0005	"	—	—	—	—	—	"	—	
	345	"	"	—	—	—	—	—	"	—	
	340	0.0004	"	—	—	—	—	—	"	—	
	340	"	"	—	—	—	—	—	"	—	
	340	0.0003	"	—	—	—	—	—	"	—	
	340	"	"	—	—	—	—	—	"	—	
	345	0.0008	"	—	—	t	ttt	ttt	S	tttt	
		U.S.A. 3									
	360	"	"	—	—	—	tt	ttt	"	tttt	
	340	0.0007	"	—	—	—	—	—	"	—	
	340	"	"	—	—	—	—	—	"	—	
	340	0.0006	"	—	—	—	—	—	"	t	
	340	"	"	—	—	—	—	—	"	—	
	355	0.0005	"	—	—	—	—	—	"	—	
	340	"	"	—	—	—	—	—	"	—	
	340	0.0004	"	—	—	—	—	—	"	—	
	340	"	"	—	—	—	—	—	"	—	
	340	0.0003	"	—	—	—	—	—	"	—	
	340	"	"	—	—	—	—	—	"	—	

The contents of one tube of dry serum were dissolved in exactly 26 c.cm. of 0.85 % NaCl solution.

Serum and toxin in contact for 1 hour at room temperature before injection.

TABLE XI. *Loss in unitage which occurs as serum ages.*

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day : 1	2	3	4	5	6	Remarks
2. 6. 11	355	0.0006 U.S.A. 3	1/800	—	S	—	—	—	—	Serum T. 2 110 units.
	365	..	1/900	—	..	—	—	t	t	
	340	..	1/1000	—	..	—	—	ttt†		
	370	..	1/1100	—	..	—	—	tt	†	
	370	..	1/1200	—	..	†				
8. 11. 12	375	..	1/700	—	..	—	—	—	—	2nd test of same serum. Now 90 units. Loss of about 20 % in 17 months.
	380	..	1/800	—	..	—	t	t	tt	
	370	..	1/900	—	..	—	ttt	†		
	360	..	1/1000	—	..	ttt	†			
	370	..	1/1100	—	..	ttt†				
9. 10. 11	385	..	1/1400	—	—	—	—	—	S t	Serum T. 3 160 units.
	385	..	1/1500	—	—	—	t	tt	.. †	
	380	..	1/1600	—	—	—	t	tt	†	
	355	..	1/1700	—	—	tt	†			
	370	..	1/1800	—	—	†				
8. 11. 12	365	..	1/1200	—	S	—	t	tt	tttt	2nd test of same serum. Now 120 units. Loss of about 25 % in 13 months.
	350	..	1/1300	—	..	ttt	†			
	355	..	1/1400	—	..	ttt	†			
	355	..	1/1500	—	..	†				
	365	..	1/1600	—	..	†				
1. 7. 12	340	..	1/1300	—	—	—	tt	tttt	tttt	Serum T. 4 150 units.
	355	..	1/1400	—	—	—	tt	tttt	†	
	340	..	1/1500	—	—	—	tttt	†		
	350	..	1/1600	—	—	t	†			
	345	..	1/1700	—	ttt	†				
8. 11. 12	365	..	1/1100	—	S	t	tt	tt	tttt	2nd test of same serum. T. 4 say 130 units. Loss of about 10 % in 4 months.
	360	..	1/1200	—	..	t	tt	ttt	tttt	
	340	..	1/1300	—	..	tt	tttt	tttt†		
	315	..	1/1400	—	..	tt	ttt†			
	340	..	1/1500	—	..	ttt	†			
30. 8. 12	370	..	1/2000	—	S	—	—	—	—	Serum T. J 20. 8. 12. 300 units.
	360	..	1/2500	—	..	—	—	—	—	
	375	..	1/3000	—	..	—	tt	ttt	†	
	360	..	1/3500	—	..	ttt	†			
	370	..	1/4000	—	..	†				
19. 3. 13	340	0.0006 U.S.A. 4	1/2000	—	—	—	†			2nd test of same serum, T.J. Now only just 200 units. Loss of 33 % in 7 months.
	350	..	1/2225	—	—	†				
	340	..	1/2500	—	—	†				
	310	..	1/2750	—	—	†				
	350	..	1/3000	—	—	†				

TABLE XI (*continued*).

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day : 1	2	3	4	5	6	Remarks
28. 1. 13	365	0.0006 U.S.A. 3	1/1500	—	—	—	—	S	—	} <i>Canterbury</i> , 14. 1. 13. 200 units.
	370	"	1/2000	—	—	—	t	"	†	
	345	"	1/2500	—	—	ttt	†			
	360	"	1/3000	—	ttt	†				
	370	"	1/3500	—	†					
10. 4. 13	340	0.0006 U.S.A. 4	1/1250	—	—	S	tt	†		} Same serum, C, 14. 1. 13. 125 units. Loss of about 37 % in 3 months.
	340	"	1/1500	—		"	†			
	340	"	1/1750	—	ttt	†				
	310	"	1/2000	—	†					
23. 1. 13	340	0.0006 U.S.A. 3	1/750	—	—	S	—	—	—	} <i>Juno</i> , 14. 1. 13. 250 units.
	370	"	1/1000	—	—	"	—	—	—	
	350	"	1/1500	—	—	"	—	—	—	
	345	"	1/2000	—	—	"	—	—	—	
	345	"	1/2500	—	—	"	ttt	†		
10. 4. 13	340	0.0006 U.S.A. 4	1/1500	—	tttt	†				} Same serum, J, 14. 1. 13. 125 units. Loss of about 50 % in 3 months.
	340	"	1/1750	—	†					
	340	"	1/2000	—	†					
	340	"	1/2500	—	†					
16. 4. 13	340	"	1/1000	—	—	—	S	—	—	}
	340	"	1/1250	—	—	—	"	—	—	
14. 5. 12	345	0.0006 U.S.A. 3	1/1500	—	—	—	—	S	—	} Serum T, C, 9. 11. 11. 200 units.
	345	"	1/2000	—	—	—	—	"	ttt	
	350	"	1/2500	—	—	ttt	†			
	375	"	1/3000	—	t	†				
	375	"	1/3500	—	ttt	†				
29. 11. 12	340	"	1/1000	—	—	—	—	—	—	} 2nd test of same serum. Still 200 units. No loss in 6 months.
	340	"	1/1500	—	—	—	—	—	—	
	345	"	1/2000	—	—	—	t	ttt	ttt	
31. 3. 13	360	0.0006 U.S.A. 4	1/1000	—	—	—	—	—	—	} 3rd test of same serum. Now 150 units. Loss of 25 % in 4 mos.
	340	"	1/1250	—	—	—	—	†	—	
	340	"	1/1500	—	—	t	ttt	†		
	350	"	1/1750	—	—	ttt	†			
	370	"	1/2000	—	—	ttt	†			
30. 10. 11	380	0.0006 U.S.A. 3	1/2000	—	—	—	—	—	—	} Serum T, J, 30. 9. 11. 350 units.
	380	"	1/2500	—	—	—	—	—	—	
	365	"	1/3000	—	—	—	—	—	—	
	375	"	1/3500	—	—	—	t	†		
	360	"	1/4000	—	t	†				

TABLE XI (continued).

Date of test	Weight of guinea-pigs	Amount of toxin (c.cm.)	Amount of serum (c.cm.)	Day : 1	2	3	4	5	6	Remarks
29. 11. 12	340	0.0006 U.S.A. 3	1/2000	—	S	—	—	—	—	2nd test of same serum. 300 units. Loss of about 15 % in 13 mos.
	355	"	1/2500	—	"	—	—	—	—	
	310	"	1/3000	—	"	—	—	t	t	
	315	"	1/3500	—	"	ttt††				
11. 8. 11	355	"	1/500	—	S	—	—	—	—	Serum T. B, 14. 7. 11. 100 units.
	365	"	1/750	—	"	—	—	—	—	
	350	"	1/1000	—	"	—	tt	ttt	ttt	
	345	"	1/1250	—	"	t †				
	345	"	1/1500	—	"	ttt†				2nd test of T. B. 14. 7. 11. Now 75 units. Loss of 25 % in 15 mos.
29. 11. 12	340	"	1/500	—	S	—	—	—	—	
	350	"	1/750	—	"	—	—	—	t	
	355	"	1/1000	—	"	t †				
29. 7. 11	310	"	1/500	S	—	—	—	—	—	Serum T. R, 27. 7. 11. 100 units.
	345	"	1/750	"	—	—	—	—	—	
	355	"	1/1000	"	—	—	—	—	—	
	355	"	1/1500	"	t †					
	355	"	1/2000	"	ttt†					2nd test of same serum, T.R. Now 75 units. Loss of 25 % in 16 mos.
29. 11. 12	370	"	1/500	—	S	—	—	—	—	
	360	"	1/750	—	"	—	—	—	t	
	350	"	1/1000	—	"	t †				
30. 8. 12	375	"	1/2000	—	S	—	—	—	—	Serum T. C. 20. 8. 12. 300 units.
	375	"	1/2500	—	"	—	—	—	—	
	375	"	1/3000	—	"	—	tt †			
	375	"	1/3500	—	"	t †				
	360	"	1/4000	—	"	ttt †				
9. 4. 13	340	0.0006 U.S.A. 4	1/1250	—	—	—	S	—	—	2nd test of same serum. Now 175 units. Loss of about 40 % in about 7 mos.
	340	"	1/1500	—	—	—	"	—	—	
	345	"	1/1750	—	—	t	"	ttt†		
	340	"	1/2000	—	—	tt	†			
	340	"	1/2250	—	t	tttt †				
1. 7. 12	345	0.0006 U.S.A. 3	1/750	—	—	—	—	—	—	Serum T. M, 19. 4. 12. 200 units.
	340	"	1/1000	—	—	—	—	—	—	
	355	"	1/1250	—	—	—	—	—	—	
	365	"	1/1500	—	—	—	—	—	?	
	375	"	1/2000	—	—	tt	ttt †			
7. 4. 13	340	0.0006 U.S.A. 4	1/1250	—	—	—	tt	tt	ttt	2nd test of same serum. Now 125 units. Loss of 40 % in 9 mos.
	350	"	1/1500	—	t	tttt †				
	310	"	1/1750	—	t	tttt†				
	340	"	1/2000	—	ttt †					

TABLE XI (continued).

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day:						Remarks
				1	2	3	4	5	6	
9. 12. 12	365	0.0006 U.S.A. 3	1/1500	—	—	—	—	—	—	Serum T. J 1. 11. 12. 200 units.
	355	"	1/2000	—	—	—	—	—	—	
	350	"	1/2500	—	t	+	—	—	—	
	380	"	1/3000	—	ttt	+	—	—	—	
	340	"	1/3500	—	+	—	—	—	—	
10. 4. 13	340	0.0006 U.S.A. 4	1/1250	—	—	S	tt	ttt	ttt	2nd test of same serum. Now 125 units. Loss of about 35 % in 4 mos.
	340	"	1/1500	—	t	+	—	—	—	
	340	"	1/1750	—	+	—	—	—	—	
	340	"	1/2000	—	+	—	—	—	—	
9. 12. 12	345	0.0006 U.S.A. 3	1/1500	—	—	—	—	—	—	Serum T. C, 1. 11. 12. 200 units.
	340	"	1/2000	—	—	—	—	?t	—	
	360	"	1/2500	—	—	t	ttt+t	—	—	
	370	"	1/3000	—	t	ttt+t	—	—	—	
	365	"	1/3500	—	ttt	+	—	—	—	
10. 4. 13	340	0.0006 U.S.A. 4	1/1250	—	—	—	tt	tt	—	2nd test of same serum. Now 125 units. Loss of about 35 % in 4 mos.
	340	"	1/1500	—	—	ttt+t	—	—	—	
	340	"	1/1750	—	—	+	—	—	—	
	345	"	1/2000	—	ttt	+	—	—	—	
20. 5. 12	370	0.0006 U.S.A. 3	1/2500	—	—	—	—	—	—	T. J, 19. 4. 12. 300 units.
	370	"	1/3000	—	—	—	—	—	—	
	340	"	1/3500	—	t	ttt+t	—	—	—	
	350	"	1/4000	—	tt	+	—	—	—	
	380	"	1/4500	—	ttt+t	—	—	—	—	
7. 4. 13	340	0.0006 U.S.A. 4	1/2250	—	—	—	—	—	—	2nd test of the same serum. Now 250 units. Loss of about 16 % in 10 mos.
	340	"	1/2500	—	—	t	ttt	tttt	+	
	340	"	1/2750	—	tt	ttt	+	—	—	
	340	"	1/3000	—	tt	+	—	—	—	

six months—a loss which one would not expect from general experience. It is therefore justifiable to presume that while Toxin 3 has deteriorated somewhat it is also probable that Toxin 4 is a little stronger than it was supposed to be. This presumption is borne out by the results of tests on samples of sera most generously given me by Dr Th. Madsen and by Prof. Paltauf.

TABLE XII.

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day : 1	2	3	4	5	6	Remarks
20. 2. 13	340	0.0006 U.S.A. 3	1/200	—	—	S	—	—	—	Copenhagen serum. On 10. 10. 11 the titre was 1.6 fach.
	365	„	1/300	—	—	„	uu†			
	310	„	1/400	—	uu†					
	340	„	1/600	—	†					
	365	„	1/800	—	†					
20. 2. 13	340	„	1/200	—	—	S	—	—	—	Copenhagen serum. On 26. 5. 11 the titre was 2.2 fach.
	340	„	1/300	—	—	„	—	—	—	
	340	„	1/400	—	—	„	—	—	—	
	340	„	1/600	—	—	„	—	—	—	
	340	„	1/800	—	—	„	—	—	—	
25. 4. 13	340	0.0006 U.S.A. 4	1/100	—	S	—	—	—	—	Copenhagen serum. On 18. 4. 12 the titre was 1.6 fach.
	310	„	1/200	—	„	tt	tt	tt	tt	
	340	„	1/300	—	„	uu†				
	340	„	1/400	—	†					
	340	„	1/500	—	†					
25. 4. 13	350	„	1/100	—	S	—	—	—	—	Copenhagen serum. On 23. 10. 12 the titre was 1.6 fach.
	345	„	1/200	—	„	—	—	—	—	
	345	„	1/300	—	„	tt	tt	tt	tt	
	340	„	1/400	—	„	†				
	340	„	1/500	—	†					
21. 4. 13	345	„	1/100	—	—	—	—	—	—	Copenhagen serum. On 1. 7. 12 the titre was 1.6 fach.
	350	„	1/200	—	—	—	—	—	—	
	355	„	1/300	—	—	—	t	t	t	
	350	„	1/400	—	uu†	†				
	340	„	1/500	—	†					
21. 4. 13	340	„	1/100	—	—	—	—	—	—	Copenhagen serum. On 4. 9. 12 the titre was 1.6 fach.
	350	„	1/200	—	—	—	—	—	—	
	340	„	1/300	—	—	tt	tt	uu	uu	
	355	„	1/400	—	uu	†				
24. 4. 13	340	„	1/100	—	—	S	—	—	—	Copenhagen serum. On 2. 2. 12 the titre was 1.6 fach.
	340	„	1/200	—	—	„	tt	tt	tt	
	340	„	1/300	—	uu	†				
	340	„	1/400	—	†					
	340	„	1/500	tt	†					
25. 4. 13	360	„	1/100	—	S	—	—	—	—	Copenhagen serum. On 9. 3. 12 the titre was 1.6 fach.
	345	„	1/200	—	„	—	—	tt	tt	
	350	„	1/300	—	„	uu†				
	340	„	1/400	—	†					
	350	„	1/500	—	†					

TABLE XII (*continued*).

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day : 1	2	3	4	5	6	Remarks
31. 3. 13	370	0.0006 U.S.A. 4	1/500	—	—	—	—	—	S	} Vienna serum. On 27. 11. 12 was 15-20 fach.
	365	"	1/750	—	—	—	—	—	" t	
	360	"	1/1000	—	—	—	—	t	" +	
	350	"	1/1250	—	—	t	+			
	365	"	1/1500	—	tt	+				
31. 3. 13	340	"	1/500	—	—	—	—	—	—	} Vienna serum. On 1. 3. 11 was 25 fach.
	340	"	1/750	—	—	tt	+			
	370	"	1/1000	—	—	tt	+			
	340	"	1/1500	—	+					
	370	"	1/2000	t	+					
31. 3. 13	340	"	1/1000	—	—	—	—	—	—	} Vienna serum. On 4. 9. 12 was 40-50 fach.
	340	"	1/1500	—	—	—	—	t	+	
	355	"	1/2000	—	—	ttt+				
	340	"	1/2500	—	—	ttt+				
	340	"	1/3000	—	+					
31. 3. 13	360	"	1/1000	—	+					} Rotterdam serum No. 150. Titre unknown.
	350	"	1/1500	—	+					
	345	"	1/2000	tt	+					
	350	"	1/2500	tt	+					
	370	"	1/3000	tt	+					
2. 4. 13	345	"	1/250	—	—	—	—	—	—	}
	380	"	1/500	—	—	—	—	—	—	
	380	"	1/750	—	—	—	—	—	—	

If we suppose for the purposes of comparison that the German unit is equal to say 40 U.S.A. units, then in the case of the Copenhagen sera we see that of the two sera tested against Toxin 3 one has practically lost nothing in 21 months and the other about 46% in 16 months, whereas the six sera tested against Toxin 4 show an average loss of some 57% in 10 months. The loss of unitage in the Vienna sera appears to be even greater. These rates of deterioration are excessive and one is forced to the conclusion that the test dose of Toxin 4 is somewhat too large under our conditions of experiment.

Now before being sent out this toxin was tested against a standard antitoxin and the L+ dose fixed only after careful experiment by the U.S.A. Public Health Service. My results have disagreed with theirs too consistently and regularly for the want of agreement to be ascribed to errors in technique on my part and the only explanation I can offer is that the resistance of guinea-pigs bred in England may be less than

that of American animals. Such a racial variation in susceptibility with regard to diphtheria is referred to by Ehrlich and by Theobald Smith. (Cf. G. Dean, p. 471, *The Types of Immunity in The Bacteriology of Diphtheria* edited by G. H. F. Nuttall and G. S. Graham Smith. Cambridge University Press 1907.)

The deterioration of Toxin 3 is easily accounted for as it was not kept under absolutely the best conditions.

It is therefore advisable that in each laboratory where this method is used a standard dry antitoxin should be kept to control the toxin from time to time and to standardize each new batch of toxin.

Provided this precaution is taken this method of standardizing tetanus antitoxin may be accepted as being just as simple, accurate and reliable as Ehrlich's method of standardizing diphtheria antitoxin.

The following experiments may be considered unnecessary. They are given just to "complete the picture."

Rosenau and Anderson recommend that the toxin and serum should be allowed to remain in contact for one hour at room temperature before injection.

From Table XIII we gather that in the case of ordinary serum this duration of contact is ample.

When, as in the case of tetanus, large doses of serum are necessary the question of the presence or absence of an antiseptic becomes of importance and it may be found to be preferable to make use of a serum which has been sterilized by filtration and heating and which contains no preservative.

The effect of heating tetanus antitoxin at 57° C. is shown in Table XIV, which also provides further evidence of the reliability of this method of testing.

We see that serum which is free from antiseptic can be heated daily for one hour for three days without loss of antitoxin.

If an antiseptic such as carbolic acid be present there is a loss of some 12% which all occurs during the first hour. We find also that tetanus antitoxin differs somewhat from diphtheria antitoxin in being affected by heat in the presence of an antiseptic even though diluted with one third of its bulk with water and with NaCl present to the extent of 1.5% (as in first stage of Gibson and Bauzhof's concentration process).

TABLE XIII. *Effect of temperature and duration of contact upon the union of toxin and antitoxin.*

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day : 1	2	3	4	5	6	Remarks
16. 4. 13	340	0.0006 U.S.A. 4	1/1750	—	—	—	S	—	—	} <i>T. 31 serum.</i>
	340	..	1/2000	—	—	—	..	—	tt	
	340	..	1/2250	—	—	tt	..	+	—	
	340	..	1/2500	—	—	ttt†	—	—	—	
	350	..	1/2750	—	tt	+	—	—	—	
	340	..	1/3000	—	ttt	+	—	—	—	} <i>T. 31 serum. Serum and toxin in contact ½ hr. at room temperature before injection.</i>
21. 4. 13	340	..	1/1500	—	—	—	—	—	—	
	345	..	1/1750	—	—	—	—	—	—	
	350	..	1/2000	—	—	—	—	—	—	
	340	..	1/2250	—	—	tt	tttt	+	—	
	340	..	1/2500	—	—	tttt	+	—	—	} Serum and toxin in contact ½ hr. at 36° C. before injection.
	340	..	1/1500	—	—	—	—	—	—	
	345	..	1/1750	—	—	—	—	—	—	
	350	..	1/2000	—	—	t	t	t	tt	
	340	..	1/2250	—	—	ttt	ttt	tttt	+	
	340	..	1/2500	—	tt	+	—	—	—	} Serum and toxin in contact 1 hr. at room temperature before injection.
	340	..	1/1500	—	—	—	—	—	—	
	340	..	1/1750	—	—	—	—	—	—	
	340	..	1/2000	—	—	—	tt	tt	tt	
	340	..	1/2250	—	—	—	tt	ttt	+	
	350	..	1/2500	—	—	ttt	+	—	—	} <i>T. 31 serum. Serum and toxin in contact 1 hr. at 36° C. before injection.</i>
	355	..	1/1500	—	—	—	—	—	—	
	340	..	1/1750	—	—	—	—	—	—	
	340	..	1/2000	—	—	—	—	—	—	
	340	..	1/2250	—	—	t	ttt	tttt	+	
	345	..	1/2500	—	—	t	ttt	tttt	+	} Serum and toxin in contact 1½ hrs. at room temperature before injection.
	340	..	1/1500	—	—	—	—	—	—	
	340	..	1/1750	—	—	—	—	—	—	
	340	..	1/2000	—	—	—	—	—	—	
	340	..	1/2250	—	—	—	—	—	t	
	340	..	1/2500	—	—	ttt	+	—	—	} Serum and toxin in contact 1½ hrs. at 36° C. before injection.
	340	..	1/1500	—	—	—	—	—	—	
	355	..	1/1750	—	—	—	—	—	—	
	340	..	1/2000	—	—	—	—	—	—	
	340	..	1/2250	—	—	t	tt	tt	—	
	340	..	1/2500	—	—	tt	+	—	—	} Serum and toxin in contact 2 hrs. at 36° C. before injection.
	345	..	1/1500	—	—	—	—	—	—	
	345	..	1/1750	—	—	—	—	—	—	
	340	..	1/2000	—	—	—	—	—	t	
	345	..	1/2250	—	—	—	tt	tttt	+	
	340	..	1/2500	—	—	ttt	+	—	—	} <i>T. 31 serum. Serum and toxin in contact 2 hrs. at 36° C. before injection.</i>
	350	..	1/1500	—	—	—	—	—	—	
	340	..	1/1750	—	—	—	—	—	—	
	345	..	1/2000	—	—	—	—	—	—	
	340	..	1/2250	—	—	—	t	tt	tt	
	340	..	1/2500	—	—	tt	tt†	—	—	

TABLE XIV. *Showing the effect of heating tetanus antitoxin at 57° C.*

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
3. 1. 13	360	0.0006	1/1500	—	S	—	—	—	—	Serum T. C without antiseptic, Unitage before heating 1 hr. × 57° C. 200 units.
	340	U.S.A. 3	1/2000	—	..	—	tt	tt†		
	365	..	1/2500	—	..	t	†			
	375	..	1/3000	—	..	†				
	375	..	1/3500	—	..†					
	350	..	1/1500	—	..	—	—	—	—	Serum T. C. Unitage after heating 200 units.
	340	..	1/2000	—	..	—	—	tt	†	
	340	..	1/2500	—	..	t	†			
	365	..	1/3000	—	..	†				
	380	..	1/3500	—	..	†				
6. 1. 13	340	..	1/1500	—	—	—	—	—	S	Serum T. J without antiseptic before heating 1 hr. × 57° C. Unitage = 150 units.
	375	..	1/2000	—	t	ttt†				
	370	..	1/2500	—	ttt†					
	365	..	1/3000	—	†					
	380	..	1/1500	—	—	—	—	—	S	Serum T. J after heating. Unitage = 150 units.
	350	..	1/2000	—	t	ttt†				
	360	..	1/2500	—	ttt†					
15. 5. 13	340	0.0006	1/1000	—	—	S	—	—		T. C serum 10. 5. 13, before heating. No antiseptic present.
	340	U.S.A. 4	1/1250	—	—	..	—	—		
	345	..	1/1500	—	—	..	—	t		
	340	..	1/1750	—	—	..	tt	ttt		
	340	..	1/2000	—	t	..†				
17. 5. 13	310	..	1/1250	S	—	—	—	—	—	Same serum after heating for 1 hr. at 57° C.
	340	..	1/1500	..	—	—	—	t	tt	
	340	..	1/1750	..	tt	ttt	ttt†			
	350	..	1/2000	..	tt	ttt†				
	345	..	1/2250	..	ttt†					
	350	..	1/1250	..	—	—	—	—	—	Same serum after heating for 1 hr. at 57° C. on two succeeding days.
	340	..	1/1500	..	—	—	—	t	tt	
	340	..	1/1750	..	—	t	ttt†			
	370	..	1/2000	..	tt	tt	†			
	350	..	1/2500	..	tt	†				
	340	..	1/1250	..	—	—	—	—	—	Same serum after heating for 1 hr. at 57° C. on three succeeding days.
	360	..	1/1500	..	—	—	—	t	tt	
	365	..	1/1750	..	t	tt	ttt	ttt†		
	340	..	1/2000	..	—	†				
	340	..	1/2500	..	ttt†					
7. 5. 13	370	..	1/1500	—	—	—	—	—	—	Serum T. 31 containing a little antiseptic. On 16. 4. 13 the unitage was 225, see Table XIII.
	345	..	1/1750	—	—	—	—	—	—	
	350	..	1/2000	—	—	—	—	†		
	340	..	1/2250	—	tt	ttt†				
	370	..	1/2500	—	ttt†					

TABLE XIV (continued).

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day: 1	2	3	4	5	6	Remarks
5. 5. 13	340	0.0006 U.S.A. 4	1/1500	—	—	—	—	t	t	T. 31 heated for 1 hr. at 57° C.
	340	..	1/1750	—	—	ttt	tttt†			
	350	..	1/2000	—	tt	†				
	370	..	1/2250	—	ttt†					
	370	..	1/2500	—	ttt†t					
7. 5. 13	340	..	1/1000	—	—	—	—	—	—	T. 31 heated for 1 hr. at 57° C. on two successive days.
	340	..	1/1500	—	—	—	—	—	—	
	340	..	1/1750	—	—	—	—	—	tt	
	340	..	1/2000	—	—	ttt†				
	340	..	1/2250	—	tt	†				
	355	..	1/1000	—	—	—	—	—	—	T. 31 heated for 1 hr. at 57° C. on three successive days.
	340	..	1/1500	—	—	t			tttt	
	345	..	1/1750	—	—	tt		†		
	360	..	1/2000	—	ttt	†				
	370	..	1/2250	—	tttt†					
5. 5. 13	350	..	1/500	—	—	—	—	—	—	Tetanus anti-toxin containing carbolic acid 0.4 %.
	350	..	1/750	—	—	—	—	—	—	
	365	..	1/1000	—	—	—	—	—	—	
	360	..	1/1250	—	—	ttt	ttt†			
	340	..	1/1500	—	tttt†					
10. 5. 13	340	..	1/500	S	—	—	—	—	—	The same serum diluted with 3rd its vol. of distilled water, NaCl added to 1½ %, and heated to 57° C. for 7 hrs.
	340	..	1/600	..	—	—	—	—	—	
	340	..	1/700	..	—	t	tt	ttt		
	340	..	1/800	..	t	ttt†t				
	340	..	1/900	..	†					

Experiments on mice.

Some experiments were performed to ascertain the minimal lethal dose of these toxins for mice.

On 18. 4. 13 the M.L.D. of Toxin 3 for mice of 30 grms. was found to be 0.000002 grm. and on 17. 4. 13 Toxin 4 had an M.L.D. for mice of the same weight of 0.0000007 grm.

On 24. 4. 13, 0.0001 grm. of Toxin 4 was mixed with varying amounts of Copenhagen serum (sample of 2. 2. 12) and injected into mice weighing 35 grm.

Two mice each of which received 0.0001 gr. tox. + 1/750 c.c. serum—both lived.

..	+ 1/1000	..	—both died in 5 days, one not of tetanus.
..	+ 1/1250	..	—both lived.
..	+ 1/1500	..	—both died on 4th day.
..	+ 1/2000	..	—both died on 2nd day.

The mice with larger doses of serum lived.

SUMMARY.

The American method of standardizing tetanus antitoxin has been studied and two samples (Toxins 3 and 4 of this paper) have been examined.

It has been found that :

1. Toxin 3 remained stable for two years under conditions which were not absolutely ideal, as the tube containing the supply was repeatedly opened during that time and the air in this neighbourhood, especially in winter, is very moist.

Toxin 4 has so far remained stable for 12 months. This toxin has been distributed in small quantities in tubes which have been exhausted and sealed in the flame.

2. By itself each of these toxins has given results consistent within the error of experiment when used to establish the M.L.D. and also to test a serum. But when the same serum was evaluated by both toxins they gave differing values. This difference in value appears to be due to

(a) Deterioration in Toxin 3 which raised the L+ dose from 0.0006—0.00075.

(b) Excess of toxicity in Toxin 4.

Whether this is due to increased susceptibility of the race of animals used for the tests can at present be only a matter of surmise.

3. The American method shows clearly that tetanus antitoxin can be heated at 57° C. for one hour on each of three successive days without loss of antitoxin provided no antiseptic (*e.g.* phenol) be present. Should such a preservative have been added then the loss on heating amounts to about 12 % and occurs during the first hour.

4. Tetanus antitoxin containing phenol appears to be more susceptible to heat than diphtheria antitoxin under the same conditions.

The general conclusion arrived at is that, provided control experiments are carried out from time to time (as in the case of standard diphtheria toxin), this method of standardizing tetanus antitoxin is—as claimed for it—simple, accurate and reliable.

ADDENDUM.

While this paper was in the printer's hands I had the opportunity of examining another sample of the dry powdered standard toxin for which I am greatly indebted to Dr J. F. Anderson. The results of these tests and of the evaluation of another market sample of antitetanic serum are given below.

It will be seen that the two toxins give practically identical results with both a strong and a weak serum and that the M. L. D. is much the same in each case.

On one occasion mentioned in the table and in several other experiments not given here the toxin was injected subcutaneously in the side over the lower ribs. It was noticed that in these cases the tetanic symptoms made their appearance earlier, but that death took place at about the same time as when the toxin was injected in the middle line of the abdomen.

Minimal lethal dose of toxins U.S.A. 4 and U.S.A. 5.

Date of test	Dose in grammes :						Remarks
	0·000007	0·000006	0·000005	0·000004	0·000003	0·000002	
U.S.A. 4.							
7. 7. 13	4	4	6	5	5	—	—
24. 10. 13	—	4	4	4	5	—	—
29. 10. 13	—	3	5	4	5	—	Injections made at side over lower ribs.
U.S.A. 5 received 6. 10. 13.							
10. 10. 13	—	4	5	5	Slight tetanus	Slight tetanus	

Testing toxins U.S.A. 4 and U.S.A. 5 against the same sera.

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day : 1	2	3	4	5	6	Remarks
9. 12. 13	365	0.0006 U.S.A. 4	1/50	—	S	—	—	—	t	} killed Serum Vulcan
	345	"	1/60	—	"	—	—	—	tt	
	340	"	1/70	—	"	—	—	t	tt	
	345	"	1/80	—	"	t	tttt	tttt†	—	
	355	"	1/90	—	"	tttt†	—	—	—	
	365	0.0006 U.S.A. 5	1/50	—	S	—	—	—	tt killed	} killed Serum Vulcan
	375	"	1/60	—	"	—	tt	tttt	† —	
	340	"	1/70	—	"	tttt†	—	—	—	
	350	"	1/80	—	"	tt	tttt†	—	—	
	350	"	1/90	—	"	†	—	—	—	
12. 11. 13	370	0.0006 U.S.A. 4	1/6000	—	—	—	S	—	—	} Serum T. R. 4
	355	"	1/7000	—	—	tt	—	†	—	
	340	"	1/8000	—	—	ttt	†	—	—	
	350	"	1/9000	—	—	tttt	†	—	—	
	340	0.0006 U.S.A. 5	1/6000	—	—	tt	S	tt	ttt killed	} Serum T. R. 4
	340	"	1/7000	—	—	ttt	†	—	—	
	340	"	1/8000	—	—	tttt	†	—	—	
	370	"	1/9000	—	—	tttt†	—	—	—	

Testing the value of Tizzoni's antitetanic serum.

Date of test	Weight of guinea-pigs	Amount of toxin (grm.)	Amount of serum (c.cm.)	Day : 1	2	3	4	5	6	Remarks
8. 11. 13	350	0.0006 U.S.A. 4	1/500	tt	†	—	—	—	—	} Market sample of serum Tizzoni 200,000 units in 5 c.cm.
	340	"	1/1000	tttt†	—	—	—	—	—	
	340	"	1/1500	tttt†	—	—	—	—	—	
	370	"	1/2000	tttt†	—	—	—	—	—	
	340	"	1/2500	tttt†	—	—	—	—	—	
	340	"	1/3000	tttt†	—	—	—	—	—	
12. 11. 13	340	"	1/50	—	—	—	S	—	—	} killed
	340	"	1/100	—	—	—	"	—	—	
	340	"	1/250	—	—	tt	"	tttt	tttt killed	

PUBLICATIONS RECEIVED.

BOOKS.

ABEL, R. (1913). *Handbuch der praktischen Hygiene*. Jena: Gustav Fischer. 25 x 17 cm. Vol. I, 808 pp., 230 text-figs.; vol. II, 458 pp., 83 text-figs. Price 24 marks unbound, 26 marks bound.

Abel's new *Handbook of Practical Hygiene* is a work of the first order in which a number of distinguished authors are responsible for the different sections. As indicated in the title the work is essentially practical. It is intended for medical men, technical experts, officials holding administrative positions, persons in every capacity who have to deal with practical health problems. For this reason the work differs materially from scientific treatises on Hygiene in the arrangement of the subject matter; debatable scientific subjects etc. are omitted and only the essential scientific data are included.

The contents may be briefly indicated as follows:

- Vol. I, pp. 1-12, "Significance and Development of Practical Hygiene," by R. Abel; pp. 13-44, "Vital Statistics," by Fr. Prinzing; pp. 45-107, "Hygiene of Sites for Towns, Suburbs, Villages etc.," by H. Chr. Nussbaum; pp. 108-164, "Hygiene of the Dwelling," by M. Versmann and H. Fürst; pp. 165-240, "Water Supply," by Salomon; pp. 241-357, "Disposal of Refuse," by J. Brix; pp. 358-384, "Burial and Burial Places etc.," by R. Abel; pp. 385-529, "Nutrition, Foods," by R. Abel; pp. 530-542, "Clothing," by W. Gehrke; pp. 543-553, "Baths," by A. Herzberg; pp. 554-579, "Physical Culture," by F. A. Schmidt; pp. 580-605, "Infection and Immunity," by F. Löffler; pp. 606-634, "General Principles for Combating Contagious Diseases," by M. Kirchner; pp. 635-808, "Combating each Individual Contagious Disease," by H. Flatten; "Tuberculosis and Smallpox" are treated by R. Abel.
- Vol. II, pp. 3-25, "Hygiene of Infancy," by G. H. Sieveking; pp. 26-34, "Hygiene of Early Childhood," by R. Abel; pp. 35-102, "School Hygiene," by G. Leubuscher; pp. 103-113, "Hygiene of Youth," by Kaup; pp. 114-240, "Industrial Hygiene," by Roth; pp. 241-321, "Hygiene of the Sick and Invalid," by E. Dietrich; pp. 322-345, "Military Hygiene," by von Vagedes; pp. 346-368, "Prison Hygiene," by E. Ziemke; pp. 369-382, "Railway Hygiene," by Tracinski; pp. 383-403, "Hygiene of Ships and Harbours," by Sanneman; pp. 404-430, "Tropical Hygiene," by C. Schilling. A large number of excellent illustrations accompany the text giving views of buildings and streets, town and hospital plans etc., etc. The work will surely appeal to a wide circle of readers. N.

- BESSON, A. (1913). *Practical Bacteriology, Microbiology and Serum Therapy*. (Medical and Veterinary.) (Translated and adapted from the 5th French edition by Dr H. J. Hutchens, D.S.O.) London: Longmans, Green & Co., 39, Paternoster Row. 892 pp., 416 illustrations (149 coloured). 24×16 cm. Price 36/- net. Cloth.

Professor Hutchens' English Edition of Dr Besson's well-known work ought to be of great service both to students and advanced workers in bacteriology. The work covers a very wide field, including not only pathogenic bacteria and allied species, but parasitic fungi, spirochaetes, protozoa belonging to various groups, and filterable viruses. The details of technique are most clearly described, the important question of differential diagnosis of allied species is carefully dealt with, many little known species are mentioned, and the uses of agglutinins, vaccines, sera, etc. considered in detail.

The book, which is well printed and contains numerous excellent illustrations, can be thoroughly recommended. G. S. G.-S.

- CASTELLANI, A. and CHALMERS, A. J. (1913). *Manual of Tropical Medicine*. Second Edition. London: Baillière, Tindall & Cox, 8, Henrietta Street, Covent Garden. xxxii+1747 pp., 15 coloured pls, 630 text-figs. 22×14 cm. Price 21/- net. Cloth.

We welcome the second edition of this well-known manual which the authors have improved by various corrections and additions besides adding considerably to the wealth of truly excellent illustrative material. It is scarcely a work which the student of tropical medicine can afford to be without. N.

- DIEUDONNÉ, A. (1913). *Immunität, Schutzimpfung und Serumtherapie*. Zusammenfassende Übersicht über die Immunitätslehre. 8th edition. Leipzig: Verlag von J. A. Barth. 248 pp., with a few text-figs. 24×17 cm. Price 6.80 marks unbound, 7.80 marks bound.

The appearance of the eighth edition of this book is the best indication that it deserves the praise we have already given to the earlier editions. Chemotherapy and Anaphylaxis are treated of in this edition. N.

- ELLIOTT, R. H. (1913). *Sclero-Corneal Trephining in the Operative Treatment of Glaucoma*. London: George Pulman & Sons, Ltd., Thayer Street, Manchester Square, W. 117 pp., 33 figs. 24×15 cm. Price 7/6 net. Cloth.

This excellent treatise is very appropriately dedicated to Priestley Smith to whom all surgeons are indebted for much of their knowledge of Glaucoma and its pathology.

After an historical introduction by Sidney Stephenson which discusses the various operations from those of Argyll Robertson to the modern procedures of Herbert, Lagrange, and others, the author's method is fully described with figures of the instruments used, and pictures of the operation as being conducted by the author in India.

The statistics are of Indian patients, and to the European surgeon it seems that Glaucoma cases in India are very numerous as compared with Europe. But with the enormous population of India, about 315 millions, it may be that there is no special tendency to the disorder, however much one may suspect that Cataract is more usual among Indians—and that lens changes may be a cause of Glaucoma in many cases.

The technique of the operation is carefully detailed with all the preliminary toilet and ritual. Reasons are given by the writer for the preference advocated for every step of his own operation.

That the procedure is very successful, at least in India, there can be no doubt, and further experience may prove that the Iridectomy operation of Von Graef may be superseded by the modern method. It appears to be more simple than that of Herbert and claims to incur less risk of accident and complication both at the time of operation and during recovery. The aim of all these Sclerotomy and Sclerectomy operations is to establish permanently a filtration or drainage between the chamber of the eye and the subconjunctival space. G. E. W.

GUITARD, E. (1913). *Deux siècles de Presse au Service de la Pharmacie et cinquante ans de "l'Union Pharmaceutique."* 2nd edition. Paris: En vente à la Pharmacie Centrale de France. 21, Rue des Nonnains-d'Hyères. 316 pp. 22 × 14 cm.

The book is divided into two parts. Part I deals with periodicals of all countries which have been of service to the pharmacist from 1665 to 1860. Part II deals with the history of the "Union Pharmaceutique" during the last 50 years. The book is published under the auspices of M. Charles Buchet, "Directeur de la Pharmacie Centrale de France," and it constitutes a valuable contribution to the history of medicine, embodying as it does the fruits of much painstaking research. Very interesting illustrations of historical documents, title pages and portraits accompany the text. N.

HAIG, K. G. (1913). *Health through Diet.* London: Methuen & Co., Ltd., 36, Essex Street, W.C. 227 pp. Price 3/6 net. Cloth.

This book gives in popular form Dr Alexander Haig's well-known views concerning uric acid, and claims to be a practical guide to the "Uric Acid Free Diet" based on eighteen years personal experience. There is no proof that the alleged benefits claimed from this diet are in any way related to its freedom from uric acid or its precursors. The diet as described here is so singularly unattractive that apart from faddists few persons are likely to be converted to its use, even if that were desirable. H. A.

HOWARD, W. L. (1913). *Plain Facts on Sex Hygiene.* London: Grant Richards, Ltd., 7, Carlton Street, S.W. 171 pp. 19 × 13 cm. Price 2/6 net. Cloth.

The author, who is an American, presents his "Plain Facts" in a forcible manner. The little book is divided into eight chapters and deals mainly with Syphilis and its baneful effects: "(1) The conditions that menace homes and children. (2) What are these diseases? (3) How the disease is contracted by the innocent. (4) Syphilis, its contagiousness. Its danger to you. (5) Hereditary Syphilis. (6) The Ghosts of Syphilis: Mental and Nervous troubles appearing late in life. (7) Syphilis in relation to Marriage. (8) The mental and physical value of continence." It is a book which contains information suited for young people of both sexes on their attaining or about to attain maturity. It can be safely recommended, it will do good in combating the crass ignorance amongst the laity. N.

— (1913). *Facts for the Married.* London: Grant Richards, Ltd., 7, Carlton Street, S.W. 161 pp. 19 × 13 cm. Price 2/6 net. Cloth.

Written in a popular vein and with a good purpose, the subject matter being divided into ten chapters or "Consultations" in which doctor and patient converse. The text is redundant in Americanisms but these do not affect the information which the book supplies and which should be in the possession of those about to marry. N.

- KNEELAND, G. J. (1913). *Commercialized Prostitution in New York City*. (Publications of the Bureau of Social Hygiene.) London: Grant Richards, Ltd., 7, Carlton Street, S.W. 334 pp. 21×14 cm. Price 7/6 net. Cloth.

The Bureau of Social Hygiene, of which Mr John D. Rockefeller, Jr. is chairman, herewith launches the first of four volumes dealing with various aspects of the problem of prostitution. The present volume contains an introduction by Mr Rockefeller, Jr. and a Supplementary Chapter by Miss Katherine B. Davis, Superintendent of the New York State Reformatory for Women. A second volume on "Prostitution in Europe" is being prepared by Mr Abraham Flexner and the other volumes will deal with Police Systems etc. The first volume is a record of a very extended and difficult investigation which has been carried out in a thoroughly scientific spirit and with the humanitarian object of combating commercialized prostitution through the knowledge which is attained. N.

- LOWRY, E. B. (1912). *Herself. Talks with Women concerning Themselves*. Chicago, U.S.A.: Forbes & Co., 443, S. Dearborn St. 221 pp. 19×13 cm. Price \$1.00 net. Cloth.

This book can be safely placed in the hands of any young woman as it gives sound advice and information in a form that is easily comprehended. It deserves every praise. N.

- (1912). *False Modesty that protects vice by ignorance*. Chicago, U.S.A.: Forbes & Co., 443, S. Dearborn St. 110 pp. 17×12 cm. Price 50 cents net. Cloth.

Upon the title page of this little book occurs a well-chosen quotation from Browning: "Ignorance is not innocence, but sin." The volume is "Dedicated to the Next Generation." It is intended for the "education of the masses to a knowledge of what is transpiring in their own communities, even in their own homes" and to bring home the facts to parents with a view to showing them "the necessity of early and proper instruction for both boys and girls in matters pertaining to sex; and to prove that the parents who withhold this knowledge are committing a crime in allowing their children to fall because of ignorance." The book is thoroughly to be recommended to parents and teachers. N.

- (1912). *Confidences. Talks with a Young Girl concerning Herself*. Chicago, U.S.A.: Forbes & Co., 443, S. Dearborn St. 94 pp. 17×12 cm. Price 50 cents net. Cloth.

This little book is intended for a girl of 10-14 to whom, the author suggests, it should be read by her mother. The subject of sex is treated in a delicate and irreproachable manner. The book should prove useful in the hands of parents and teachers. N.

- (1912). *Truths. Talks with a Boy concerning Himself*. Chicago, U.S.A.: Forbes & Co., 443, S. Dearborn St. 95 pp. 17×12 cm. Price 50 cents net. Cloth.

A little book written in a style which can be readily understood by a boy of 12-15. It gives information on sexual matters in a manner very suitable for boys and it can be safely placed in their hands by the most fastidious parent.

But for a few Americanisms the booklet is well written. N.

- MENSE, C. (1913). *Handbuch der Tropenkrankheiten*. 1 Bd. 2 Auflage. Leipzig: Verlag von J. A. Barth. 295 pp., 12 pls. (10 coloured), 200 text-figs. 27×19 cm. Price 16.20 marks unbound, 18 marks bound.

The first volume of the second edition of this excellent handbook relates to the Arthropods which cause or convey disease. The bulk of the volume (pp. 1-262) is from the pen of Dr Adolf Eysell, whilst pp. 263-283, dealing with *Phlebotomus*, are written by R. Doerr and V. Russ. This is the best work that has yet appeared on the subject of Arthropods from the medical standpoint, as it collects together a large amount of new material. The book is richly illustrated, many of the figures being original. We can but warmly commend the book to the attention of our readers. N.

- PFEIFFER, H. (1913). *Das Problem des Verbrühungstodes*. Studie zur Pathologie und Pathogenese der Thermischen Allgemeinschädigung. Wien: Ed. Hölzels Verlag, Separatkont. 272 pp., 39 curves, 4 pls. 24×17 cm. Price 8 marks unbound.

Professor Hermann Pfeiffer, who holds the Chair of General and Experimental Pathology at Graz, gives us the best and most complete treatise hitherto published on the causation of death through burns and the pathological conditions which follow burns. Judging from the bibliography, English authors have contributed but little to the subject. The book not only summarizes all that is known on the pathology of burns, it includes a considerable amount of original matter obtained in the course of experimental work and the author's extended observations over several years. His conclusions will interest all who are interested in the subject. N.

- PRESCOTT, S. C. and WINSLOW, C. E. A. (1913). *Elements of Water Bacteriology with special reference to Sanitary Water Analysis*. Third edition, rewritten. First thousand. New York: John Wiley & Sons, Inc. 318 pp. 20×13 cm. Price 7/6 net. Cloth.

That this work has reached a third edition since 1904 is a sufficient guarantee as to its usefulness. The authors are pupils of W. T. Sedgwick, S. C. Prescott being Associate Professor of Industrial Microbiology (Mass. Inst. Techn.) and C. E. A. Winslow, Associate Professor of Biology (New York). The book has been thoroughly brought up to date and can be warmly recommended to those who have to occupy themselves with the bacteriology of water. Pp. 281-306 contain a good bibliography. The book is printed in an excellent manner. N.

- PRICE, G. M. (1913). *Handbook on Sanitation*. Third Edition. New York: John Wiley & Sons, Inc.; London: Chapman & Hall, Ltd. 353 pp., 24 text-figs. 19×13 cm. Price 6/6 net. Cloth.

The subtitle of this book reads: "A Manual of theoretical and practical Sanitation. For Students and Physicians; for Health, Sanitary, Tenement-House, Plumbing, Factory, Food, and other Inspectors; as well as for Candidates for all Municipal Sanitary Positions." The book is of an elementary

description and intended for American readers, being specially suitable for Inspectors. It should serve the purpose for which it is intended. N.

- PROUT, W. T. (1913). *Lessons on Elementary Hygiene and Sanitation with special reference to the Tropics*. Third Edition. London: J. & A. Churchill, 7, Great Marlborough Street. 184 pp., 60 figs. 22 × 14 cm. Price 2/6 net. Cloth.

This book contains 17 lessons given in the form of simple lectures and is intended for use in schools situated in tropical countries. The author, who is a Medical Adviser to the Colonial Office etc., has had considerable experience of the tropics. The choice of figures has not been entirely fortunate, that of *Ankylostoma* (p. 110), for instance, being very misleading. The book may well be recommended not only for schools but for the perusal of laymen who wish to acquire some knowledge on the subjects of which it treats. N.

- ROSS, E. H. (1913). *The Reduction of Domestic Flies*. London: John Murray, Albemarle Street, W. 103 pp., with illustrations. 23 × 15 cm. Price 5/- net. Cloth.

A short account of flies, their biology and destruction. Written in a popular vein. None of the 18 illustrations are original. The book presents a very attractive appearance. N.

- RUBNER, M. (1913). Die Ernährungsphysiologie der Hefezelle bei alkoholischer Gärung. *Arch. f. Anat. u. Physiol.* (Physiologische Abteilung. 1912. Supplement-Band). 396 pp., 40 text-figures. 24 × 16 cm.

This publication has appeared as a supplementary volume to the Archives but may well be regarded as an independent book. It treats exhaustively of the subject stated in the title and incorporates a great amount of original research of the first order. N.

- STEPHENSON, S. (1913). *Eye-strain in Everyday Practice*. London: The Ophthalmoscope Press, 24-26, Thayer Street, W. 139 pp. 22 × 15 cm. Price 3/6 net. Cloth.

This is a practical and useful book on the subject, and does not claim, as in some American writings, that eye-strain causes chorea, epilepsy and insomnia. A fair consideration is given to the signs and symptoms of eye-strain as headache, blepharitis, and forms of migraine.

Perhaps it is hardly necessary to mention (on page 120) for the second time that when there is inequality in acuteness of vision the pain or discomfort is felt usually in the better or eye. This is already mentioned on page 20. The same striking story is told twice about a myopic undergraduate who was "enabled to marry" after being fitted with correcting glasses (pages 18 and 117). But the little story is worth telling especially as myopia (at any rate when of 4 D) does not so frequently lead to symptoms of eye-strain as do the other errors of refraction.

It is noteworthy that Sir Lauder Brunton considers that 80 or 90 per cent. of all headaches are due to ocular causes. G. E. W.

- STURROCK, W. D. (1913). *First Principles of Hygiene*. Oxford: The Clarendon Press. 256 pp., 47 figs. 19 × 13 cm. Price 2/6 net. Cloth.

A small book obviously intended for use in schools but written in a manner suited to the intelligent lay reader. The book should fulfil the useful purpose for which it is intended. N.

- THRESH, J. C. (1913). *The Examination of Waters and Water Supplies*. Second Edition. London: J. & A. Churchill, 7, Great Marlborough Street. 644 pp., 53 illustrations. 24×15 cm. Price 18/- net. Cloth.

This edition is an extension and amplification of the previous one. The examination of sources of water supply is first considered and then the various chemical, bacteriological and other methods of examining water. The author discusses very clearly the value of the *B. coli* group, streptococci, etc. as indices of contamination, and the importance to be attached to the constituents found in water. The chapters on chemical and bacteriological analyses are very complete, and the various problems in relation to infection are fully dealt with. The index is full, and the book can be most thoroughly recommended to all those who are interested in water supplies. G. S. G.-S.

- VERRELLS, H. V. (1912). *Experimental Hygiene*. London: Blackie & Son, Ltd., 50, Old Bailey. 147 pp., 30 figs. 18×12 cm. Price 2/- net. Cloth.

According to the preface, this book is based on the requirements of the Syllabus in Practical Hygiene issued by the Incorporated Institute of Hygiene, London. It may be found useful for such examinations, for the directions as to the experiments are clear and precise. But these experiments imply a knowledge of the elementary laws and facts of physics, chemistry and physiological chemistry; and if the student has no such knowledge, they become empirical and mechanical and they will have little or no educational value. J. E. P.

BROCHURES.

- CAPDEVILA, R. (1913). *Les Infections du premier âge*. Thèse, Paris 12. xi. 1913. Avignon: Imprimerie Rullière Frères. Rue Collège-du-Roure. 200 pp., 2 pls., 20 figs. 25×16 cm.

- The Deaf*. Handbook compiled by the National Bureau for promoting the General Welfare of the Deaf (1913), 104, High Holborn, London, W. London: P. S. King & Son., Orchard House, Westminster, S.W. 75 pp. Price 6d. net.

The handbook deals with statistics, and gives descriptions of Schools, Missions, Hospitals, Charities and other Institutions for the Deaf. As stated by Leo Bonn, President of the Bureau, in the preface, this is the first attempt at a complete directory of the kind and it should prove indispensable to all who are interested in the deaf and their welfare in the United Kingdom. x.

- GERRARD, P. N. (1913). *On the Hygienic Management of Labour in the Tropics*. An Essay (with which is incorporated the Labour Code by Courtesy of the Federated Malay States Gov't). Singapore: Methodist Publishing House. 80 pp. 25×15 cm. Cloth.

Dr Gerrard's brochure deals in a discursive style with the medical and hygienic management of coolies and coolie lines on estates. The essay is intended for the planters of Malaya and contains a number of large (folded) plans for the construction of coolie lines, latrines, wells, etc. in the tropics. A brief account is given of the various affections from which coolies may suffer and a good deal of sound advice is given from which planters and others might well reap profit. x.

- GREENWOOD, A. (1913). *The Health and Physique of School Children*. London: P. S. King & Son, Orchard Street, Westminster, S.W. 21×14 cm. 96 pp. Price 1/- net. Paper.

This brochure is published under the Ratan Tata Foundation, University of London. The Foundation has been instituted for the promotion of the study and prevention of poverty and destitution. The results of researches carried on under the Foundation will be published periodically.

The author's treatise is a scientific statistical study regarding the health and physique of school children, about one-seventh of the children (800,000) attending the elementary schools in England and Wales being included in Mr Greenwood's figures. N.

- HAFFKINE, W. M. (1913). *Protective inoculation against Cholera*. Calcutta: Thacker, Spink & Co. 98 pp., with 4 photographs. 26×18 cm.

This treatise deals with (1) the preparation of anti-cholera vaccine, (2) immunization of man against cholera, (3) anti-cholera vaccine after its devitalisation. N.

- HERMS, W. B. (1913). *A Laboratory Guide to the Study of Parasitology*. New York: The Macmillan Company. 72 pp. 22×14 cm. Price 3/6 net. Cloth.

This book serves as a guide in the practical courses of Parasitology given by the author in the University of California and may prove useful to others contemplating the undertaking of similar courses. N.

- HOLMES, J. D. E. (1913). *A Description of the Imperial Bacteriological Laboratory, Muktesar: its Work and Products*. Calcutta: Superintendent Government Printing, India. 47 pp., boards, with many photographs. 23×18 cm. Price 9d.

In this brochure, Major Holmes, Imperial Bacteriologist, gives an account of the history of the laboratory, the site and buildings, the research work conducted there, and the products of the laboratory in the matter of serums and vaccines. The report is of interest as showing the great variety of work that falls upon the responsible head of the great establishment at Muktesar. N.

- KLING, C. and LEVADITI, C. (1913). *Études sur la Poliomyélite Aiguë Épidémique*. (Publication de l'Institut Pasteur de Paris.) Paris: Imprimerie de la Cour d'Appel. 1, Rue Cassette. 124 pp., 1 pl. (coloured), 2 maps, with several text-figs. 25×16 cm.

An important contribution.

- NATTAN-LARRIER, L. (1913). *Notice sur les Travaux Scientifiques du Dr Louis Nattan-LARRIER*. Paris: Imprimerie de la Cour d'Appel. 1, Rue Cassette. 165 pp., 78 figs. 26×19 cm.

Gives a succinct account of the varied work of this talented investigator.

- WANKLYN, W. McC. (1913). *The Administrative Control of Smallpox. How to Prevent or Stop an Outbreak*. London: Longmans, Green & Co., 39, Paternoster Row. 83 pp. 23×14 cm. Price 3/6 net. Cloth.

This short treatise is intended as a companion volume to the author's *How to Diagnose Smallpox*. It deals with practical problems and is intended for D.P.H. students and those who may have to deal with outbreaks of smallpox. It is written in an easy conversational manner. N.

- ZIEMANN, H. (1913). *Zur Pathogenese, Diagnose und Prophylaxe der Tuberkulose in den Tropen. Habilitationsschrift z. Erlangung d. venia legendi in der Medizinischen Fakultät d. Friedrich Wilhelms-Universität zu Berlin.* Jena: Gustav Fischer. 23 pp.

REPORTS.

- ALLEN, H. B. (III. 1913). *Report on Health Conditions at Panama.* Commonwealth of Australia. Melbourne, Australia: McCarron, Bird & Co., Printers and Publishers, Collins Street. 27 pp., 1 map, 16 figs.
- Annual Report of the Surgeon-General of the Public Health Service of the United States for the fiscal year 1912. (1913.) Washington: Government Printing Office. 261 pp. 23×15 cm. Cloth.
- BARTOW, E. (25. III. 1912). Chemical and Biological Survey of the Waters of Illinois. Report for the Year ending December 31, 1911. (Forming Univ. of Illinois Bulletin, Vol. XI, No. 20.) 173 pp. Illinois: Published by the University, Urbana.
- EDER, M. D. (1913). *Fourth Report of the Deptford Health Centre.* London: P. S. King & Son, Orchard House, Westminster. 39 pp. Price 3d. net.
- Eleventh Report (30. XI. 1912) of the Home for the Training in Speech of Deaf Children before they are of School Age, 2201, Belmont Avenue, Philadelphia, Pennsylvania, U.S.A. (Twentieth Anniversary.) Philadelphia: 28 pp., with photographs.
- HOPE, E. W. (1913). *Report on the Health of the City of Liverpool during 1912.* Liverpool: C. Tinsling & Co., Ltd., Printing Contractors, 53, Victoria Street. 300 pp., with plates and tables. 25×16 cm. Cloth.
- HOPE, J. W. (1913). *Report on the Medical, Health, Factories, and Early Closing Department for the year ending 31st December, 1912.* Perth: A. Curtis, Acting Government Printer. 98 pp., with figs. 33×21 cm.
- HORN, A. E. and MAYER, T. F. G. (1913). *Report on certain outbreaks of Yellow Fever in West Africa in 1910 and 1911.* (Government Publication.) London: Waterlow & Son, Ltd., London Wall. 108 pp., 6 maps. 33×21 cm.
- HOUSTON, A. C. (1913). Ninth Research Report on search for certain Pathogenic Microbes in Raw River Water and in Crude Sewage. *Ninth Research Report. Metropolitan Water Board.* London. 26 pp., 3 diagrams. 33×21 cm.
- (1913). Report on the results of the Chemical and Bacteriological Examination of the London Waters for the Twelve Months ended 31st March, 1913. *Seventh Annual Report. Metropolitan Water Board.* London. 64 pp. 33×21 cm.
- LISTON, W. G. (1913). *Report of the Bombay Bacteriological Laboratory for the Year 1912.* Bombay: Printed at the Government Central Press. 39 pp. 33×21 cm. Price 5a. or 6d.
- Medical and Surgical Reports of the Buffalo General Hospital, 1913, vol. I.* Buffalo, New York, U.S.A. Hausauer-Jones Printing Co. 249 pp. 23×15 cm.
- Contains 37 papers by members of the staff, mostly surgical, with some excellent illustrations. s.
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- Seventh Report (I. IV. 1913) of the Henry Phipps Institute for the Study, Treatment, and Prevention of Tuberculosis.* (University of Pennsylvania.) Philadelphia: Henry Phipps Institute. 7th and Lombard Streets. 26×17 cm.
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NEW JOURNALS.

The American Journal of Tropical Diseases and Preventive Medicine. (Official Organ of The American Society of Tropical Medicine.) Edited by Drs Creighton Wellman, Chas. Chassignac, and Isadore Dyer. Vol. I. 25×17 cm. Published monthly by the American Journ. of Trop. Dis. Co., Ltd., New Orleans. Subscription \$2.00 a year in the U.S.A., \$2.50 a year abroad. All communications to be addressed to P. O. Drawer 602, New Orleans, La., U.S.A.

The new journal will publish original papers, especially such as deal with tropical and subtropical disease-control. The first number appeared in July 1913 and four numbers, containing 342 pages, have reached us to date. Of the original papers we may mention Chamberlain: Some features of the physiological activity of Americans in the Philippines.—Knab: The species of *Anopheles* that transmit human malaria.—Tenney: Intestinal Parasites in the Philippine Islands.—Johus: *Trypanosoma americanum* in naturally infected animals (with coloured plate).—Matas: The surgical treatment of elephantiasis etc. Notes on the society's transactions, short memoranda and book reviews conclude No. 1. The new journal will be useful in bringing together much American work that has hitherto been scattered through many journals. N.

Quarterly of the Federation of State Medical Boards of the United States. Edited by Dr Otto V. Huffman. Vol. I, No. 1. (October, 1913.) Price 2 dollars a year, or 50 cents a copy. Published quarterly by The Federation Press, Eaton, Pennsylvania.

The recently founded federation of state medical boards has for one of its objects the obtaining and publishing of accurate knowledge regarding the standards of medical education throughout the world, with the object of raising and reforming the standards and requirements. The opening number of the new periodical contains eight papers dealing with state boards, examination methods, laws, etc. and the preparation for the professions, editorials, excerpts, notes and data relating to the federation and its members. N.

The Indian Journal of Medical Research. Edited by the Director-General, Indian Medical Service, and the Sanitary Commissioner with the Government of India. Published quarterly by the Indian Research Fund Association. Vol. I, No. 1, 211 pp., 14 pls., and numerous charts. Price 10 shillings per annum, single numbers 3 shillings, including postage. Calcutta: Thacker, Spink & Co. 24×19 cm.

This new journal is an important undertaking. As the official organ of the Indian Research Fund it will receive papers, official and non-official, dealing with research in medical and sanitary science. It will take the place of *Paludism* and the *Scientific Memoirs*, consequently there is no reason to complain that one journal more has appeared to load our bookshelves. The Journal will actually fill a very long felt want in India. Besides original papers it will publish résumés of work dealing with special diseases and give summaries of interesting official reports. It must be owing to the support of the Fund that the subscription to the Journal only amounts to 10 shillings. Papers for publication should be sent to the Secretary, Scientific Advisory Board, Indian Research Fund Association, Simla. Contributors receive 50 reprints free.

The first number contains 16 contributions. Two by W. S. Patton and F. W. Cragg on new species of flies (*Musca, Philaematomyia*); two by F. M. Howlett on *Phlebotomus* and life histories of biting insects; four by E. D. W. Greig on cholera; a résumé on dysentery by J. Cunningham. A. C. MacGilchrist writes on the haemolytic action of quinine and the treatment of blackwater; H. W. Acton and R. Knowles on the diagnosis of latent malaria; C. Donovan and also W. S. Patton on Kala-azar; H. W. Acton and R. Knowles on the specific gravity of the blood and also on Kurloff bodies; C. Strickland on Malayan mosquitoes.

Excellent illustrations accompany the text. N.

Memoirs of the Department of Agriculture in India. Veterinary Series. From the Agricultural Research Institute, Pusa. Published for the Imperial Dept. of Agriculture in India by Thacker, Spink & Co., Calcutta, and W. Thacker & Co., 2, Creek Lane, London.

Vol. I, Parts 1-3, 24×18 cm. 176 pp. Parts sold separately at Rs. 2, 1-4, and 1 respectively depending on size. Vol. II, No. 1, 31 pp. Price Rs. 1. The four Nos. received, are dated January, February, April and August (1913) respectively.

Part 1 contains "Anaphylaxis in the Larger Animals" by J. D. E. Holmes; Part 2 "Salvarsan in the treatment of Surra in horses, dogs and rabbits" by J. D. E. Holmes; Part 3 "Some more successful experiments on the treatment of Surra in the camel with recommendations for systematic treatment"

by A. J. Leese. Vol. II, No. 1 contains "Some cases of Surra treated in the field and in the laboratory during the autumn of 1911" by J. D. E. Holmes. Judging from the character of the papers they contain, the Memoirs will be welcomed by many readers. N.

PERIODICAL PUBLICATIONS.

The Proceedings of the Second All-India Sanitary Conference held at Madras, November 11th to 16th, 1912. Simla (1913): Government Central Branch Press. Vols. I-IV, 33×21 cm. Boards. Vol. I, General Proceedings and Resolutions, 129 pp., frontispiece (plate), price Rs. 2-0-0; Vol. II, Hygiene, 567 pp., price Rs. 2; Vol. III, Research, 315 pp., price Rs. 2; Vol. IV, Engineering, 91 pp., 2 pls., price Rs. 2.

An important conference: Vol. I contents relate to town planning, congested areas and building laws; travelling dispensaries; plague; sanitary engineering; urban water supplies; water analysis; cholera; fevers and infectious diseases; sewage and refuse disposal; milk supply, etc., etc.

Vol. II contains 49 papers by various authors dealing with subjects included in the foregoing list, all being included under Hygiene.

Vol. III contains 31 papers by different authors dealing chiefly with plague, cholera, fevers and infectious diseases.

Vol. IV contains 11 papers dealing with (1, 2) Description of the Madras City Drainage Works, (3) The Simla Hydro-Electric Scheme, (4) Sanitary Problems in Madras, (5) Differences between English and Indian Sanitary Engineering Practice, (6) Sanitation in India, (7, 8) Dust prevention, (9) Experimental Sand and Mechanical Filters, King Institute, Guindy, (10) Madras City Water-Supply, (11) Water-Supply of Conjeevaram. N.

Proceedings of the Third Meeting of the General Malaria Committee held at Madras, November 18, 19 and 20, 1912. Simla: Government Central Branch Press. 289 pp., 3 maps, 2 tables. 33×21 cm. Boards.

Contains a large number of original papers dealing with malaria in all its aspects as well as with other insect transmitted diseases. N.

Circular of the School for Health Officers, Harvard University and Massachusetts Institute of Technology. Vol. I, No. 1. (September, 1913.) Catalogue and Announcement. U.S. America: School for Health Officers, 240, Longwood Avenue, Boston, Mass. 41 pp.

The British Guiana Medical Annual for 1911. Edited by Dr K. S. Wise. (Eighteenth year of issue.) 77+xxxi pp. Price 5/-. Demerara: Printed by "The Argosy" Co., Ltd. 21×14 cm.

Contains nine papers dealing with Narcotics and stimulants of the Guianese Indians, Trichosporosis nodosa, Tinea cruris, Cough, Leprosy treatment (Nastin and Benzoyl chloride), Enteric, Bacillus violaceus in water and milk, Milk supply in Brit. Guiana, Myiasis. N.

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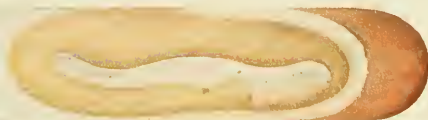
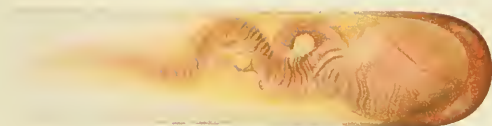
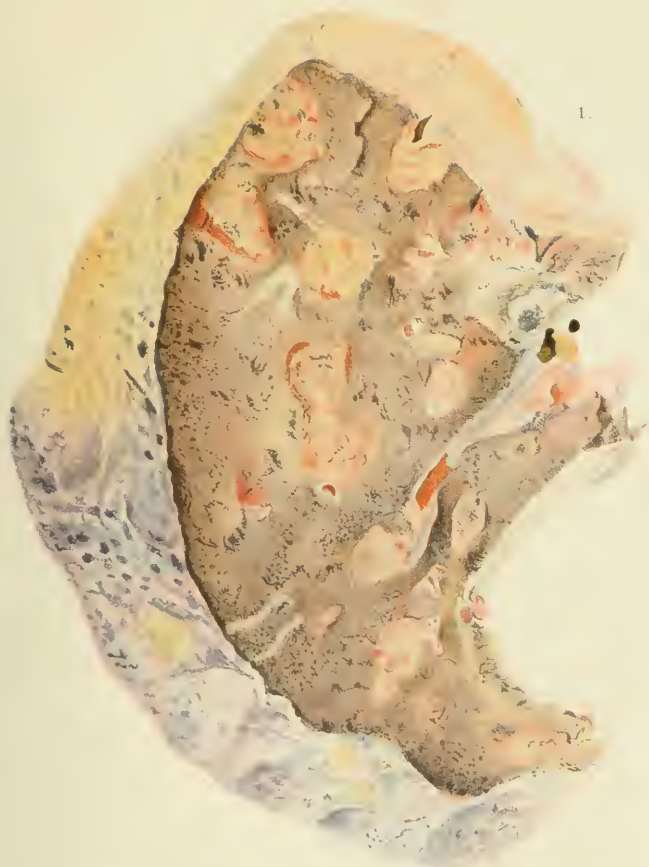




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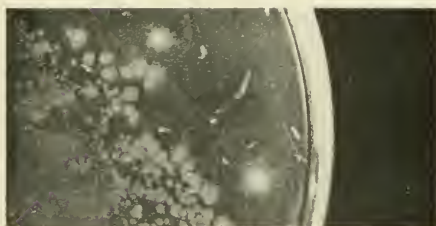


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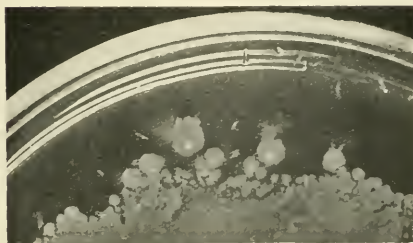


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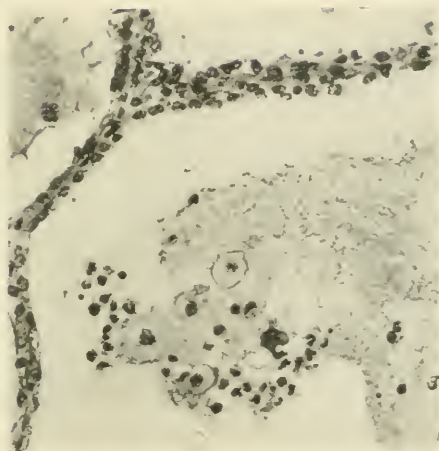


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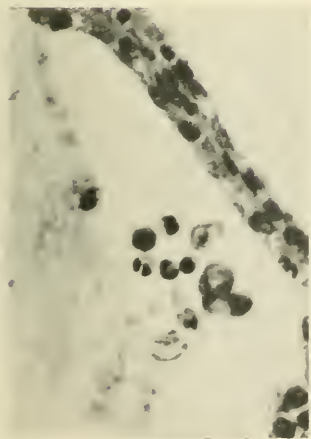


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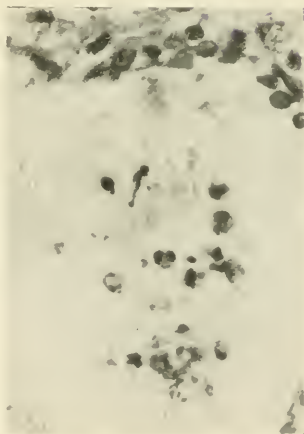


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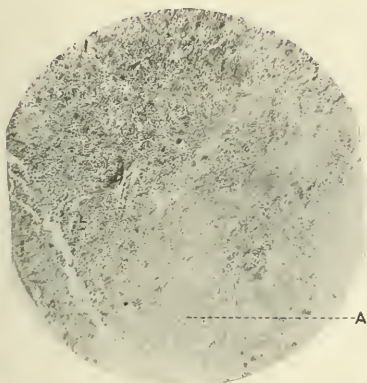


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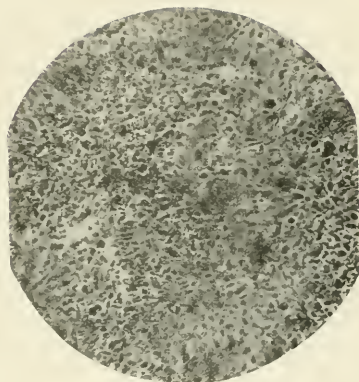


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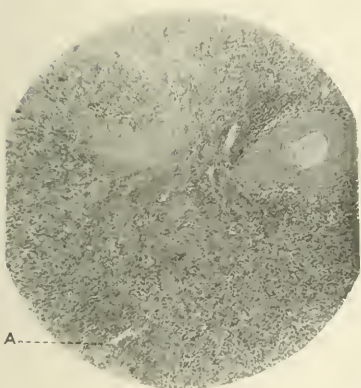


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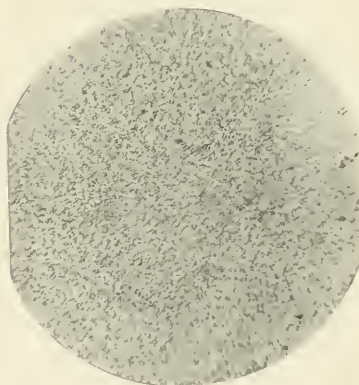


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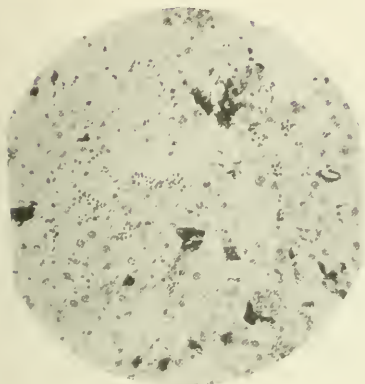


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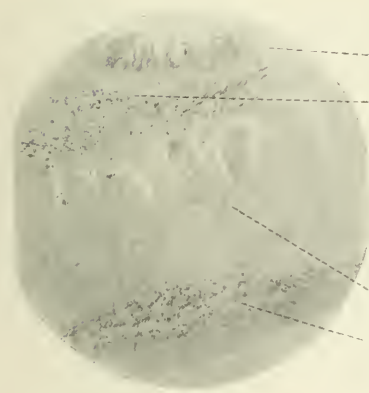


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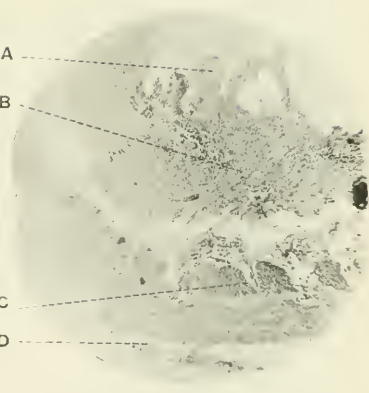


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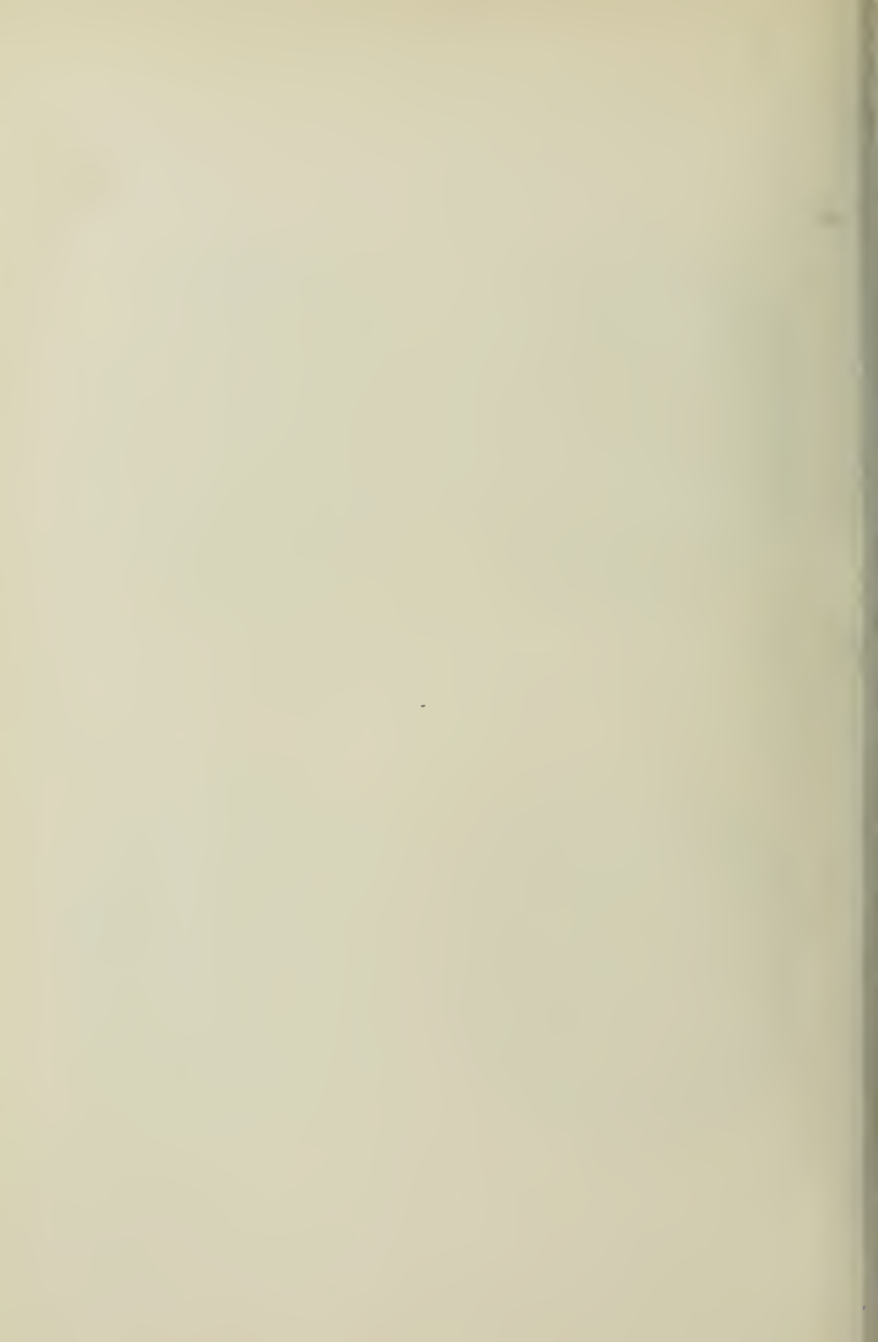




Fig. 1. Some of the inns at Manchouli. The structure seen on the right is the entrance to one of the underground type.



Fig. 2. Interior of an underground inn at Manchouli showing lower tier of berths. To take this photograph, it was necessary to lie at full length on one of the upper berths.



Fig. 3. The Chinese and Russian Expeditions. Borsja (Siberia), July 22nd-29th, 1911.
Two of the railway cars used by us are shown in the photograph.



Fig. 4. A Mongol family and hut. Charbada.



Fig. 5. The Tarbagan. The long claws are well seen.



Fig. 6. The Tarbagan. Note the small ears and sharp lower incisors.



Fig. 7. The Tarbagan. Side view.
Note the fierce appearance.



Fig. 8. A dead Tarbagan showing measurements.
Tip of nose to tip of tail = 65 cm. = 25½ ins.
,, ,, base ,, = 42 cm. = 16½ ins.
Wt. = 10 E. lbs.



Fig. 9. Skull of Tarbagan as seen from above and laterally.



Fig. 10. The Tarbagan regions. Arbulak (Siberia).
Note the mounds or "bootans" against the skyline.



Fig. 11. The Tarbagan regions near Manchouli. Note the large mounds (against skyline) and the smaller ones in front.



Fig. 12. Entrance to a newly made Tarbagan burrow. Tschintansk (Siberia). Note the absence of grass around the burrow. The ground was level and sandy.



Fig. 13. Entrance to a Tarbagan burrow situated on the slope of the hill shown on Fig. 10. Arabulak. Note rocky nature of ground and faeces on the bare space to the left of burrow.



Fig. 14. Two entrances to an "Earth." Arabulak. Note the long grass on part overhanging the burrows.



Fig. 15. Tarbagan burrow opened. No. A. Aug. 19th, 1911. Near Manchouli.

In this burrow some old Tarbagan skeletons were found.

- 1 The hat indicates entrance 2. 2 indicates blind terminus. 3 indicates nest.
4 indicates enlarged space containing faecal matter. 5 sloping entrance 1.



Fig. 16. Tarbagan burrow opened. No. B. Aug. 19th, 1911. Near Manchouli.

- 1 blind end. 2 indicates burrow going upwards. 3 enlarged space in which lies the nest, indicated by handkerchief. 4 the hat indicates sloping entrance.



Fig. 17. Tarbagan burrow No. B traced further. Aug. 21st, 1911.
Note its great depth.



Fig. 18. Tarbagan burrow opened in March, 1911 (winter), partially filled up.

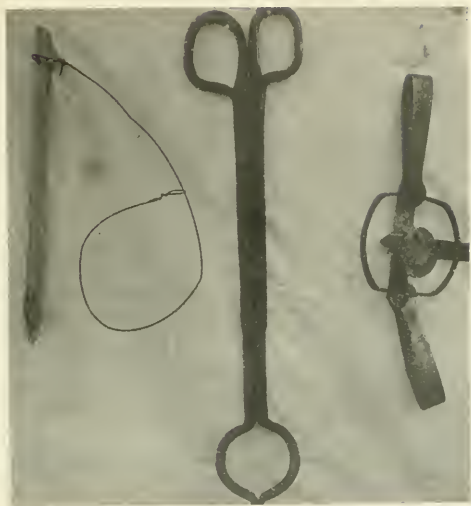


Fig. 19. On the left is a single snare with wooden peg. On the right is a strong trap (rarely used). In the middle, the special forceps.



Fig. 20. Tarbagan cages. Manchouli. Aug. 14th-Sept. 30th, 1911.
Note various types.



Fig. 21. Taking rectal temperature of Tarbagan. Manchouli.



Fig. 22. *Ceratophyllus silanticus* Wagner. The Tarbagan flea.

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